AR201-14022

To: NCIC OPPT/DC/USEPA/US@EPA, Rtk Chem/DC/USEPA/US@EPA
cc:

Subject: Submission of Test Plan and Robust Summaries for Estragole by the Flavor and Fragrance High Production Volume Consortia

Dear Ms. Whitman: On behalf of the High Production Flavor and Fragrance High Production Volume Consortia (FFHPVC), I am submitting the submission letter, test plan, and robust summaries for estragole in pdf. file format. If you have any problems with the transmission of the electronic form of these documents, please contact me at any time.

Best regards,
Timothy Adams, Ph.D.
Technical Contact Person for FFHPVC

 - Robust Summaries for Estragole.ZIP

The Flavor and Fragrance High Production Volume Consortia (FFHPVC)

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Tel. (202)-293-5800 Fax (202)-463-8998

October 21, 2002

Christie Todd Whitman, Administrator
US EPA
P.O. Box 1473
Merrifield, VA 22116
Attn: Chemical Right-to-Know Program

Dear Ms. Whitman:

On behalf or the member companies of the Terpene Consortium, the Flavor and Fragrance High Production Volume Consortia is pleased to submit the Test Plan and Robust Summaries for the chemical designated "Estragole" to the HPV Challenge Program, AR-201. The Terpene Consortium has chosen not to belong to the HPV Tracker System for submission of test plans and robust summaries. We are therefore submitting the test plan and accompanying robust summaries directly to EPA to make available to the public.

This submission includes one electronic copy in .pdf format. Hard copy can be provided upon request. The EPA registration number for the Terpene Consortium is .

Please feel free to contact me with any questions or comments you might have concerning the submission at tadams@therobertsgroup.net, tadams@cheminfox.com or 202-331-2325.

Sincerely,

Timothy Adams, Ph.D.
Technical Contact Person for FFHPVC

AR201-14022A

The Flavor and Fragrance High Production Volume Consortia

The Terpene Consortium

Test Plan for Estragole

Estragole **CAS No. 140-67-0**

FFHPVC Terpene Consortium Registration Number

Submitted to the EPA under the HPV Challenge Program by:

The Flavor and Fragrance High Production Volume Chemical Consortia

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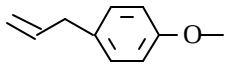
The Flavor and Fragrance High Production Volume Consortia

Test Plan for Estragole

1 IDENTITY OF SUBSTANCE



Estragole

CAS No. 140-67-0

Synonyms:

p-Allylanisole
Benzene, 1-methoxy-4-(2-propenyl)-
Chavicol methyl ether
Isoanethole
p-Methoxyallylbenzene
1-Methoxy-4-(2-propen-1-yl)benzene

2 CATEGORY ANALYSIS

2.1 INTRODUCTION

In October of 1999, members of the U.S. flavor and fragrance industries as well as other manufacturers that produce source materials used in flavors and fragrances formed consortia of companies in order to participate in the Chemical Right-to-Know Program. Members of these consortia are committed to assuring the human and environmental safety of substances used in flavor and fragrance products. The consortia are organized as the Flavor and Fragrance High Production Volume Consortia (FFHPVC). The terpene consortium, as a member of FFHPVC, serves as an industry consortium to coordinate testing activities for terpene substances under the Chemical Right-to-Know Program. Twenty-one (21) companies are current members of the Terpene Consortium. The Terpene Consortium and its member companies are committed to assembling and reviewing available test data, developing and providing test plans for each of the sponsored chemicals, and where needed, conducting additional testing. The test plan, category analysis and robust summaries presented represent the first phase of the Consortium's commitment to the Chemical Right-to-Know Program.

2.2 BACKGROUND INFORMATION

This category analysis and test plan provides data for estragole. Estragole is currently permitted by the U.S. Food and Drug Administration (FDA) for direct addition to food for human consumption as a flavoring substance and is considered by the Flavor and Extract Manufacturers' Association (FEMA) Expert Panel to be "generally recognized as safe" (GRAS) for its intended use as a flavoring substance [Hall and Oser, 1965]. Estragole occurs naturally in more than 39 foods [CIVO-TNO, 2000]. Exposure to estragole occurs principally though consumption of spices such as tarragon and essential oils derived from spices. Estragole is also added directly to food as a flavouring substance. Estragole modifies spice flavors and seasonings for condiments and meats. It is also used in heavy fruit, root beer, and anise-type

flavors. The estimated poundage of estragole added directly as a flavoring substance was reported to be approximately 500 kg [Lucas *et al.*, 1999].

Major sources of oral exposure occur *via* intake of basil, tarragon, anise, and bitter fennel. Greater than 90% of the mean daily *per capita* intake (1.0 micrograms/kg bw per day) of estragole is derived from consumption of tarragon, basil, fennel, anise and their essential oils. Based on the conservative assumption that only 10% of the U.S. population consumed foods containing estragole, the estimated daily *per capita* intake ("eaters only") of estragole from all sources is less than 10 micrograms/kg bw per day.

2.3 STRUCTURAL CLASSIFICATION

Estragole is 4-methoxyallylbenzene. Estragole is a C_{10} terpene that is recognized chemically as 4-methoxyallylbenzene. As a terpene derivative it is closely related in structure to other naturally occurring plant constituents containing a 4-alkoxyallylbenzene nucleus. Methyl eugenol (3,4-dimethoxyallylbenzene), elemicin (3,4,5-trimethoxyallylbenzene), myristicin (3-methoxy-4,5-methylenedioxyallylbenzene), and safrole (4,5-methylenedioxyallylbenzene) are all examples of *p*-alkoxyallylbenzene derivatives that can be found in spices such as nutmeg and basil. The only structural difference between estragole and these other alkoxyallylbenzene derivatives is the presence of additional ring alkoxy substituents (*i.e.*, methyl eugenol has a second ring methoxy group). *p*-Alkoxyallylbenzene derivatives participate in the same primary pathways of absorption, metabolism and excretion and exhibit the similar toxicologic endpoints (*i.e.* liver). Therefore, key data on *p*-alkoxyallylbenzene derivatives provide a more comprehensive chemical, biological and toxicological characterization of estragole.

Another structurally related substance is anethole. The structures of estragole and anethole (CAS No. 104-46-1) differ only in the position of the side-chain double bond. Estragole is 4-(2-propenyl)anisole while anethole is 4-(1-propenyl)anisole. Their similar physical properties reflect the small difference in chemical structure. The presence of an allyl side chain versus a 1-

propenyl side chain has an impact on the animal metabolism of each substance at high levels of exposure. Both substances are primarily detoxicated *via* *O*-demethylation at low levels of exposure (see below and the Test Plan for Anethole). At higher intake levels (greater than 50 to 100 mg/kg bw), estragole participates, to a significant extent, in a metabolic pathway (1'-hydrolylation) that, upon repeated daily exposure, is associated with hepatic toxicity. At these higher levels of intake, anethole mainly participates in a detoxication pathway (oxidative cleavage to yield a benzoic acid derivative) (see below). Therefore, human health toxicity data on anethole are considered relevant to estragole only in studies in which both substances participate in common pathways of metabolic detoxication (*e.g.*, *O*-demethylation) (see section 2.5 below).

2.4 INDUSTRIAL AND BIOGENIC PRODUCTION

The vast majority of estragole used as a flavoring agent in food is isolated from exotic (Reunion-type) basil that can contain as much as 90% estragole in the essential oil. Production of estragole from this source and other essential oils is approximately 10 metric tons annually [Bauer and Garbe, 1985]. However, the vast majority of estragole isolated from nature is as a component of crude sulfate turpentine (CST). Fractions containing estragole, anethole, and caryophyllene account for 1-2% of commonly distilled CST [Derefer and Traynor, 1992]. Although this represents only a small portion of CST, the sheer volume of production of CST on an annual basis provides the majority of estragole used for commercial purposes in food flavors, fragrances, cosmetics, and household products. Crude sulfate turpentine is fractionated into an anethole/caryophyllene mixture (0.5-1%) and an azetropic mixture of estragole and *alpha*-terpineol (1%). The majority of estragole present in this mixture is catalytically isomerized to anethole by the action of potassium hydroxide. The resulting mixture of anethole (mainly *trans*-anethole) and *alpha*-terpineol is further separated by fractional crystallization [Bauer and Garbe, 1985]. The majority of estragole isolated from CST, is converted to *trans*-anethole.

In 1977, it was reported that the annual production of CST in the United States was 92,750 tons (185,500,000 pounds). Based on the annual volume of production of CST and the

estragole content in CST (1%), it can be estimated that the potential amount of estragole isolated from CST is 1,855,000 pounds or 843,000 kg (843 metric tons).

Level III fugacity calculations indicate that, in the environment, estragole partitions mainly to the soil and water with less than 1% passing into the atmosphere. In the atmosphere, the relatively small amount of estragole rapidly reacts (half-life equals 3.9 hours) with hydroxyl radicals, ozone and nitrate radicals [Mackay, 1996a, 1996b]. Of more than 50 volatile organic compounds emitted by vegetation into the atmosphere, estragole was classified as exhibiting a relatively high rate of reactivity with hydroxyl radicals [Atkinson, 1990]. If it were conservatively assumed that 2% of industrially separated estragole is lost during industrial processing of CST, the vast amount (16.8 metric tons) would partition to the soil and water while the total annual estragole emitted into the atmospheric emission would be insignificant (0.17 metric tons). Compared to the amount (10 metric tons) consumed as a constituent of a traditional diet, exposure to industrial estragole loss to the atmosphere is insignificant. As a plant terpene, estragole is a normal component of the earth's atmosphere [Guenther *et al*., 1995]. However, in the absence of quantitative data on the emission rates of estragole from vegetation, it is not currently possible to estimate its annual rate biogenic production.

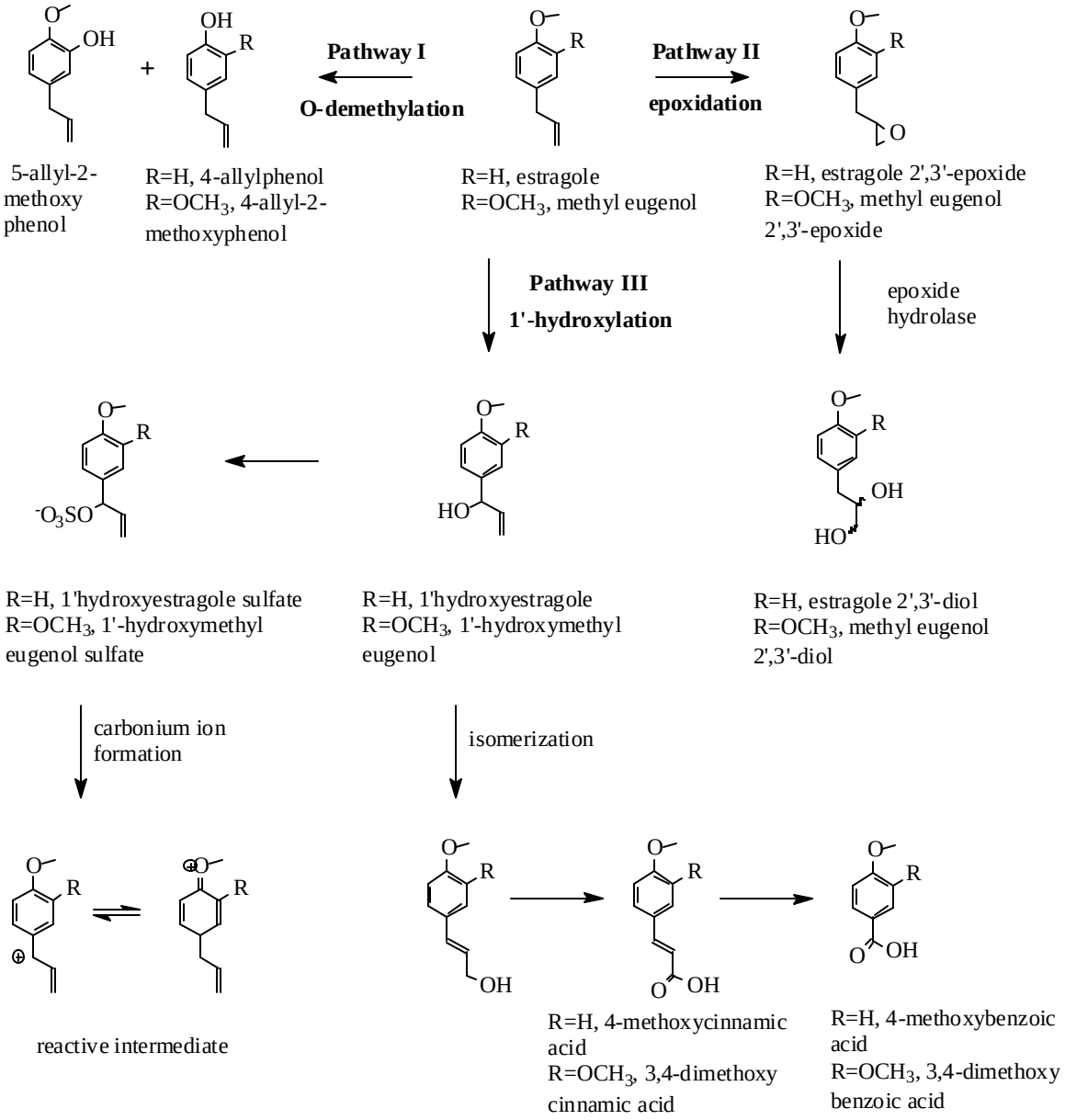
2.5 CHEMICAL REACTIVITY AND METABOLISM

The pharmacokinetic and metabolic pathways of estragole and methyl eugenol have been extensively reviewed in a recent publication (see Figure 1) [Smith *et al*., 2002]. Estragole undergoes rapid and essentially complete absorption *via* the oral route [Anthony *et al*., 1987; Sutton *et al*., 1985]. The metabolism of estragole and structurally related substances (*i.e*. methyl eugenol and *trans*-anethole) is clearly dose dependent. At low dose, (less than 10 mg/kg bw) estragole is primarily *O*-demethylated to yield the corresponding phenol derivative that is conjugated with glucuronic acid or sulfate and excreted mainly in the urine. Minor metabolic options at low levels of exposure include epoxidation of allyl double bond or 1'-hydroxylation at the benzylic position of the allyl side chain. As the dose is increased (0.05 to 1000 mg/kg bw) in

mice and rats, the *O*-demethylation pathway 1'-hydroxylation becomes saturated [Zangouras *et al*., 1981; Sangster *et al*., 1987]. The 1'-hydroxylation pathway has been shown to be a significant metabolic activation pathway leading to hepatotoxic effects in mice and rats [Miller *et al*., 1983; Phillips *et al*. 1981; Swanson *et al*., 1981; Wiseman *et al*., 1985]. Epoxidation of the allyl side chain yields a 2,3-epoxide that is detoxicated to the corresponding diol by epoxide hydrolase (EH) or to the corresponding mercapturic acid derivative by glutathione transferase (GST). Intoxication *via* the epoxidation of the allyl side chain is not as significant as activation *via* the 1'-hydroxylation pathway [Luo and Guenthner, 1995, 1996].

Intoxication *via* the 1'-hydroxylation pathway relies on formation of the labile sulfate conjugate (See Figure 1). The unstable sulfate ester hydrolyzes to form a reactive electrophilic intermediate (carbonium ion or quinonium cation) that binds hepatic proteins and hepatic DNA. The formation of protein and DNA adducts is dose-dependent [Drinkwater *et al.*, 1976; Miller *et al.*, 1982, 1983; Swanson *et al.*, 1981; Boberg *et al.*, 1983; Gardner *et al.*, 1995, 1996]. Studies on the inhibition of the sulfate conjugation pathway [Boberg *et al.*, 1983] and *in vivo-in vitro* unscheduled DNA synthesis (UDS) assays of estragole or methyl eugenol and their 1'-hydroxy metabolites [Chan and Caldwell, 1992; Caldwell *et al.*, 1992] provide substantial evidence that the sulfate ester of the 1'-hydroxy metabolite is the principal hepatotoxic metabolite in animals.

Figure 1
Metabolism of Estragole in Animals



*Excerpted from Smith *et al*., 2002

harmacokinetic Data

Approximately 70% of a 100 microgram dose of ^{14}C-methoxy-labeled estragole given by gelatin capsule to humans (2) was recovered within 48 hours, the majority of which was recovered in the urine (35% eliminated after 8 hours, 49.4% after 24 hours, and 61.2% after 48 hours), and remainder recovered in expired air (greater than 11% eliminated after 8 hours) [Sangster *et al.*, 1987].

In nine fasted human volunteers, ingestion of ginger snap cookies containing approximately 216 micrograms methyl eugenol (3.7 micrograms/kg bw) resulted in peak serum concentrations of 25-100 pg/g (approximately 0.000025-0.00010 micrograms/ml) with a mean of 16 pg/g [Masten, 2000]. A similar mean serum level (24 pg/g) was measured in 209 adults reported in an NHANES III U.S. survey. Over 98% of those surveyed containing detectable levels of methyl eugenol [Barr *et al*., 2000].

Serum levels in humans are 10,000 times less than those measured in mice and rats exposed to intoxicating levels of methyl eugenol [NTP, 2000; Graves and Runyon, 1995].

Greater than 95% of a single dose of 200 mg/kg bw of methyl eugenol or 56-66% of a dose of 100 mg/kg bw of estragole administered to male rats *via* gavage was excreted in the urine within 24 hours [Solheim and Scheline, 1973]. When the same dose levels were administered by intraperitoneal injection, greater than 85% of the methyl eugenol dose and 77-87% of the estragole dose were excreted in the urine after 24 hours [Solheim and Scheline, 1973].

In female rats, greater than 71% of a 50 mg/kg bw oral dose of ^{14}C-methoxy labeled estragole was eliminated in the first 24 hours with an additional 3.5% eliminated in the next 24 hours. Approximately 1% remained in the carcass at 48 hours. Approximately 38% was eliminated in the urine, 31% in expired air, and 1.3% in the feces [Zangouras, 1982]. In a dose-dependent

toxicokinetic study, female Wistar rats were given dose levels of 0.05 to 1000 mg/kg bw of ^{14}C-estragole. At the low doses (0.05 to 50 mg/kg bw), the majority (55% on day 1 and 2.7% on day 2) of the dose was eliminated as ^{14}C-labeled CO_2 in expired air. Urinary elimination accounted for a total 32.5% of the total radioactivity after 2 days. At higher dose levels (500 and 1000 mg/kg bw), elimination of radioactivity *via* expired air was less (29% on day 1 and 17% on day 2) and urinary elimination was greater (30% on day 1 and 29% on day 2) indicating a changeover in metabolism and elimination [Anthony *et al.*, 1987].

Rats administered either 37 or 150 mg/kg bw oral dose of methyl eugenol achieved peak plasma levels of 1.5 and 4 micrograms/ml. Plasma half-lives for methyl eugenol were 30 to 60 minutes and the area under the curve (AUC) was 97 and 225 micrograms/ml/minute at 37 and 150 mg/kg bw, respectively [Graves and Runyon, 1995].

F344/N rats (12/sex/group) were given 37 mg/kg bw by intravenous injection or 37, 75, or 150 mg/kg bw of methyl eugenol by oral intubation and blood was collected at time points up to 360 minutes [NTP, 2000]. Maximum plasma concentrations (C_{MAX}) of 0.656 to 3.84 micrograms/ml for males and 1.14 to 8.25 micrograms/ml for females were proportional to oral dose levels. Time to maximum plasma levels (T_{MAX}) was rapid (5 minutes) and independent of dose. The AUC increased linearly with dose for both males and females. The AUCs were in the range of 33.5 to 459 micrograms/ml/minute for males and 27 to 307 micrograms/ml/minute for females. Percent bioavailability also increased with dose. Bioavailability of methyl eugenol after a single oral dose was low (6% at 37 mg/kg bw and 19% at 150 and 300 mg/kg bw). Disappearance of half-lives were in the range from 60-115 minutes for both sexes. Seventy-two (72) hours after oral or intravenous administration of [^{14}C]-methyl eugenol to male rats, radioactivity was concentrated mainly in the liver (liver/blood ratio, 2-3) [NTP, 2000]. In mice given 25, 50, or 75 mg/kg bw, peak plasma levels were similar to those for rats (0.38 - 3.10 micrograms/ml for males and 0.12 - 4.4 micrograms/ml for females) and were reached in 5 minutes (T_{MAX}) in all groups except females in the 25 mg/kg bw groups which showed T_{MAX} of 15 minutes. Plasma half-lives were shorter (30 minutes) and the AUCs were significantly lower

than those recorded for rats (4.91-48.4 micrograms/ml/minute for males and 3.27-60.5 micrograms/ml/minute for females) indicating that methyl eugenol was eliminated more rapidly from the mouse.

In a second toxicokinetic study of longer duration [NTP, 2000], the pharmacokinetic profile was followed during repeated oral administration to rats and mice. Blood was taken from F344/N rats that had been treated with 37, 75, 150, or 300 mg/kg bw of methyl eugenol by gavage daily, 5 days per week for 6, 12, or 18 months. B6C3F1 mice treated at the same dose levels were monitored at 12 and 18 months. Absorption was extremely rapid in all dosed groups. Time to C_{MAX} was less than 5 minutes. Elimination from the blood was also rapid with elimination half-lives of 1-2 hours in both sexes. At 6 months, peak plasma levels (C_{MAX}) increased with increasing dose for most groups. Female concentrations (1.4-2.4 micrograms/ml) were higher than males (0.5-0.4 micrograms/ml) at the two lowest doses, but male concentrations (1.3-4.0 micrograms/ml) were higher than those (0.8-3.1 micrograms/ml) of females at the two highest doses. Generally, at the same dose levels, C_{MAX} was lower after 6 months of daily exposure than after single dose administration suggesting increased ability to metabolism methyl eugenol. Significant increases in both C_{MAX} and AUC between 6 and 12 months in the 150 and 300 mg/kg bw groups is evidence that metabolic saturation is achieved after prolonged exposure at higher dose levels. At all dose levels, females showed the AUC similar to naïve animals while males at 37, 75 and 150 mg/kg bw exhibited increased AUC suggesting enzymatic induction plays a more important role in males. An increase in the AUC with time suggests a decrease in the capacity to metabolize methyl eugenol with age [NTP, 2000].

For mice given 35, 75, or 150 mg/kg bw per day for 2 years, absorption was also rapid. C_{MAX} was reached after 5 minutes and increased with increasing dose for both male and females. Elimination half-lives increased with dose suggesting that the elimination was saturated for both sexes [NTP, 2000].

Male Fisher F344/N rats were given a single dose of 118 mg/kg bw [ring-^{14}C]-methyl eugenol and blood and urine were collected regularly and analyzed. Greater than 72% was eliminated in the urine, 13% in the feces, and less than 0.1% in expired air after 72 hours. Minute amounts (less than 0.4%) remained in the tissue at 72 hours with the majority being present in the liver. In female mice given the same dose, 85% was eliminated in the urine, 6% in the feces, less than 0.1% in the expired air, and less than 0.3% in the tissue. The largest amount was found in the fat, followed by the muscle and liver [Burkey *et al.*, 1999].

Based on the above data, it may be concluded that estragole and methyl eugenol are rapidly absorbed by the oral route and metabolized in the liver. Compared to female rats, male rats are more prone to experience metabolic saturation after prolonged (greater than 6 months), exposure to high dose levels of methyl eugenol. Male rats also experience metabolic induction at lower dose levels and earlier in exposure than do female rats.

In rodents and in humans, routes of elimination at low dose include loss of carbon dioxide *via* expired air (*i.e.*, arising from *O*-demethylation) and excretion of polar metabolites in the urine. At higher dose levels the fraction eliminated by expired air decreases while the fraction of non-volatile urinary metabolites increases.

2.5.1 Metabolism

Approximately 39% and 46% of a 100 mg/kg bw dose of estragole given to rats by the oral or intraperitoneal route, respectively, is present in the 48-hour pooled urine as the *O*-demethylation metabolite 4-allylphenol (See Figure 1). Other metabolites accounting for 17% of the oral dose or 31% of the intraperitoneal dose include the product of epoxidation, hydration and subsequent oxidation of the terminal alcohol (3-hydroxy-3-(4-methyoxyphenyl)propionic acid) of the allyl side-chain and the products of alkene isomerization, oxidation of the resulting C_3 position, and *beta*-oxidation yielding 4-methoxybenzoic acid and 4-methoxyhippuric acid. Approximately 5-

10% of the dose was excreted as the 1'-hydroxylation metabolite, 1'-hydroxyestragole [Solheim and Scheline, 1973].

A single intraperitoneal injection of 200 mg/kg bw of estragole, methyl eugenol, or safrole was given to male Wistar rats and urine was collected every 2 hours for 24 hours. Twenty-four (24) hours after treatment animals were terminated and the livers were removed. Urinary metabolites included the epoxide of the parent substance and the epoxide of the O-dealkylated metabolite (*i.e.*, *p*-allylcatechol epoxide from methyl eugenol and safrole and *p*-allylphenol epoxide from estragole). Liver homogenates showed the presence of safrole epoxide metabolites but not those of methyl eugenol or estragole. Liver microsomal preparations show the presence of the epoxide metabolite identified in the urine for all three substances [Delaforge *et al.*, 1980].

Twenty-one day old mice were given 185 micromoles/100 g bw of either estragole or safrole by intraperitoneal injection and the urine was analyzed for 1'-hydroxy metabolites 24 hours later. The dose level corresponds to 274 mg/kg bw of estragole and 300 mg/kg bw of safrole. Approximately 23% of estragole and 12% of the safrole was recovered from the 24-hour urine as the corresponding 1'-hydroxy metabolite, whereas, adult male mice (9-12 weeks) excreted up to 46% of the 300 mg/kg bw intraperitoneal dose of safrole as 1'-hydroxysafrole [Drinkwater *et al.*, 1976].

Formation of the 1'-hydroxy metabolite has been shown to be dose-dependent in both mice and rats [Zangouras *et al.*, 1981]. A dose-dependent increase in the urinary excretion of the glucuronic acid conjugate of 1'-hydroxyestragole occurs when dose levels of 0.05, 5, 500, 1,000 mg/kg bw of [^{14}C-methoxy]-estragole is administered orally to rats or by intraperitoneal injection to mice. Only 0.9% of the dose is excreted in the urine of rats given 0.05 mg/kg bw while 8.0% is found at 1,000 mg/kg bw. The total production and exposure to the 1'-hydroxy metabolite increased significantly (1,224 to 255,000 nmoles/kg per day) as the dose was increased from 5 to 500 mg/kg. Conversely, the same increase in dose resulted in a decrease in

O-demethylation from approximately 40% to 20% in both mice and rats. Thus, an increase in dose and a shift in metabolic pathways produce a marked increase in exposure to the 1'-hydroxy metabolite.

At low dose in humans, the 1'-hydroxylation pathway is of minor importance. Two male volunteers fed a gelatin capsule containing 100 micrograms [methoxy-^{14}C]-estragole (1.5 micrograms/kg bw) excrete the bulk (72% and 67%) of the radioactivity in the urine and as exhaled CO_2 within 48 hours. Principal metabolites included those derived from *O*-demethylation and oxidative degradation of the allyl side chain (i.e., 4-methyoxyhippuric acid, the glycine conjugate of 4-methoxycinnamic acid, and 4-methoxyphenyllactic acid). Urinary 1'-hydroxyestragole accounted for approximately 0.3% of the total dose [Sangster *et al.*, 1987]. The importance of *O*-demethylation pathway at low dose levels in human has also been observed for the double bond isomer, 4-propenylmethoxybenzene (anethole) [Sangster *et al.*, 1987; Caldwell and Sutton, 1988; Newberne *et al.,* 1999].

The 1'-hydroxylation pathway in rat and human liver microsomes indicate that the reaction is catalyzed predominantly by CYP2E1 and probably CYP2C6. The rate of 1'-hydroxylation of methyl eugenol varied widely in 13 human liver microsome samples (37 fold), but the highest activities in humans were similar to the activities in control rat liver microsomes [Gardner *et al.*, 1997]. Inducers of CYP-450 increased the number of methyl-eugenol-protein adducts. Auto-induction of the 1'-hydroxylation pathway was reported in hepatic microsomes of rats given 30-300 mg/kg bw per day oral doses of methyl eugenol for 5 days but not in rats given 10 mg/kg bw per day for 5 days [Gardner *et al.*, 1997].

In summary, *O*-demethylation is the principal detoxication pathway at low dose. At low dose levels, humans, mice, and rats show a similar tendency to metabolize alkoxyallylbenzene derivatives (*e.g.* estragole) by *O*-demethylation. At low dose significant amounts of estragole or methyl eugenol are *O*-demethylated, but as dose levels increase 1'-hydroxylation and

epoxidation of alkoxyallylbenzene derivatives (*e.g*. estragole) increase. Human production of 1'-hydroxy metabolite is expected to be very low levels of exposure (100 micrograms or 1.5 micrograms/kg bw) given that urinary excretion of the 1'-hydroxy metabolite accounts for less than 0.5% of urinary metabolites [Zangouras *et al*. 1981; Anthony *et al*., 1987].

2.6 SUMMARY FOR CATEGORY ANALYSIS

At low levels of exposure, estragole undergoes metabolic detoxication primarily *via* *O*-demethylation to yield the corresponding phenol derivative that is readily excreted as the glucuronic acid or sulfate conjugate in the urine. As dose levels increase, a switch in metabolism occurs in which an intoxication 1'-hydroxylation pathway competes favorably with the detoxication *O*-demethylation pathway. Under these high-dose conditions liver toxicity is normally observed in animal studies.

3 TEST PLAN

3.1 CHEMICAL AND PHYSICAL PROPERTIES

3.1.1 Melting Point

The calculated melting point for estragole has been reported to be -1.19 °C (adapted Stein and Brown method) [MPBPVP EPI Suite, 2000].

3.1.2 Boiling Point

The measured boiling point of estragole has been reported to be 216°C at 764 mm Hg [Merck Index, 1998] and 216°C at 760 mm Hg [Fragrance Materials Association]. The calculated boiling point according to the MPBPWIN program was 209.93°C at 760 mm Hg [MPBPVP EPI Suite, 2000]. Based on the consistency of these values, the boiling point of estragole is 216°C.

3.1.3 Vapor Pressure

Experimental value for vapor pressure was reported to be 1 mm Hg at 52.6°C [Stull, 1947]. The calculated vapor pressure of estragole has been reported to be 0.09 mm Hg (12 Pa) at 20°C [Fragrance Materials Association]. The vapor pressure of the isomer *trans*-anethole has been reported to be 0.05 mm Hg (6.67 Pa) at 20°C [FMA] and 0.041 (5.45 Pa) at 21°C for anethole, isomer unspecified [Daubert and Danner, 1989]. Given that the structure of estragole and anethole differ only in the position of a side-chain double bond, similar vapor pressures are expected at 20°C. Therefore, the vapor pressure of estragole is approximately 0.09 mm Hg (12 Pa) at 20°C.

3.1.4 n-Octanol/Water Partition Coefficients

The Log KOW was calculated resulting in a value of 3.47 [KOWWIN EPI Suite, 2000] for estragole, in good agreement with the log KOW of 3.39 [KOWWIN EPI Suite, 2000] and 3.11 [Interactive Analysis LogP and LogW Predictor] reported for the isomer anethole.

3.1.5 Water Solubility

The solubility of estragole in an experimental study was reported to be 178 mg/L at 25°C [WSKOWIN EPI Suite, 2000a (Yalkowski, S.H. and Dannenfelser, R.M., 1992)]. The calculated value based on the log KOW of 3.47 was reported to be 84.55 mg/L at 25°C [WSKOWIN EPI Suite, 2000b]. The water solubility of the double bond isomer anethole was reported to be 111 mg/L at 25°C that is in good agreement with the measured value for estragole [WSKOWIN EPI Suite, 2000a (Yalkowski and Dannenfelser, 1992)].

3.1.6 New Testing Required

None.

3.2 ENVIRONMENTAL FATE AND PATHWAYS

3.2.1 Photodegradation

The calculated half-life value for estragole has been reported to be 2.36 hours [AOPWIN EPI Suite, 2000]. The short half-life of estragole is expected based on the fact that the 1'-position of the side chain is both a benzylic and an allylic position. This position is a site for rapid hydrogen abstraction by hydroxy radicals, peroxide radicals, and nitrogen dioxide radicals. Of more than 50 volatile organic compounds emitted by vegetation into the atmosphere, estragole was classified as exhibiting a relatively high rate of reactivity with hydroxyl radicals (no robust summary prepared) [Atkinson, 1990].

3.2.2 Stability In Water

No hydrolysis is possible for estragole. Estragole is expected to be stable in aqueous solution.

3.2.3 Biodegradation

The isomer of estragole, anethole, exhibited ready and ultimate biodegradability as measured by carbon dioxide production in an OECD 301B Guideline study. Anethole (mixed isomers) was 91% degraded within 28 days [Quest International Inc., 1994]. Based on model predictions [BIOWIN EPI Suite, 2000] estragole is anticipated to be ultimately biodegradable. Although model predictions and data available for the isomer, anethole, predict that estragole should be readily biodegradable, it is recommended that estragole be subjected to a biodegradability study according to a standard OECD Guideline protocol.

3.2.4 Fugacity

Transport and distribution in the environment were modeled using Level III Fugacity-based Environmental Equilibrium Partitioning Model through the EPA EPI Suite 2000 program. The principal input parameters into the model are molecular weight (148.20), melting point (-1.19 °C), vapor pressure (0.09 mm Hg), water solubility (178 mg/L at 20 °C), and log Kow (3.47). The model predicts that estragole is distributed mainly to the soil (78.8%) and water (19.7%) with less than 1% passing into the atmosphere [Mackay, 1996a, 1996b].

The significance of these calculations must be evaluated in light of the fact that estragole is a product of plant biosynthesis. Therefore, the environment produces estragole. The model does not account for the influence of biogenic production on partitioning in the environment nor does it take into account any biodegradation.

3.2.5 New Testing Required

- Biodegradation study of estragole according to a standard OECD Guideline protocol.

3.3 ECOTOXICITY

3.3.1 Acute Toxicity to Fish

A measured LC50 is available for the *p*-alkoxyallyl derivative, methyl eugenol. In rainbow trout and bluegill sunfish, the 96-hour LC50 for methyl eugenol was determined to be 6 mg/L (95% C.I. 4.9-7.2 mg/L) and 8.1 mg/L (95% C.I. 7.4-9.0 mg/L), respectively [Beroza *et al*., 1975]. The acute 96-hour LC50 of anethole in fathead minnows using a continuous flow method was reported to be 7.69 mg/L [Broderius *et al.*, 1990]. Additionally, a calculated LC50 is available for estragole. The calculated 96-hour LC50 is 4.561 mg/L [ECOSAR EPI Suite, 2000].

Although the data for methyl eugenol, anethole and estragole consistently show an LC50 value of 5-10 mg/L, given the animal toxicity of estragole at high dose level, it is suggested that an LC50 be performed for estragole using a standard OECD Guideline 203 protocol.

3.3.2 Acute Toxicity to Aquatic Invertebrates

An OECD Guideline 202-I study is available for estragon oil (tarragon oil), the principal component of which is estragole (70-88%) (no robust summary for Lawrence, 1994). The 48-hour EC50 was 30.5 mg/L in *Daphnia magna* [Barth and Winkler, 2001]. The calculated 48-hour LC50 for estragole in *Daphnia magna* was reported to be 5.410 mg/L [ECOSAR EPI Suite, 2000]. This is in good agreement with an experimental 48-hour LC50 of 6.80 determined for *Daphnia magna* exposed to the 1-propenyl isomer, anethole [Broderius *et al.*, 1990].

3.3.3 Acute Toxicity to Aquatic Plants

The 96-hour IC50 experimental value for green algae for the structurally related substance *trans*-anethole was reported to be 9.571 mg/L [Broderius *et al*., 1990]. The calculated 96-hour EC50 for estragole in green algae was reported to be 3.681 mg/L [ECOSAR, EPI Suite,

2000]. Although the experimental IC50 value for the isomer is in good agreement with the calculated EC50 value for estragole, the calculated data should be further validated by comparison to an experimental EC50 determined for estragole. Therefore, an acute toxicity study is recommended using an OECD Guideline 202 protocol.

3.3.4 New Testing Required

Based on the current ecotoxicity database, the following studies are recommended:

- An acute toxicity study for fish using an OECD Guideline 203 protocol
- An acute toxicity study for algae using an OECD Guideline 201 protocol

3.4 HUMAN HEALTH TOXICITY

3.4.1 Acute Toxicity

In rats and mice, estragole showed low oral acute toxicity with oral LD50s of 1,230-1,820 mg/kg bw for rats and 1,250 mg/kg bw for mice. Low acute dermal toxicity is reflected in an LD50 value of greater than 5,000 mg/kg bw for rabbits [Moreno, 1972a, 1972b; Jenner *et al*., 1964].

Given the numerous studies available, additional acute toxicity tests in mammals are not recommended.

3.4.2 *In vitro* and *In vivo* Genotoxicity

3.4.2.1 In vitro

Extensive *in vitro* assays have been conducted on estragole and its metabolites. Estragole was negative in common strains of *Salmonella typhimurium* with and without metabolic activation [Zani *et al.*, 1991; Zeiger *et al.*, 1987; Sekizawa and Shibamoto, 1982; To *et al.*, 1982; Dorange *et al.*, 1977]. In one study [To *et al.*, 1982], a significant increase in the revertants per plate was reported for strain TA1538 in the presence of S-9 and 3'-phosphoadenosine 5'-phosphosulfate (PAPS) cofactor. The authors proposed that mutagenic response was related to the formation of the sulfate ester of an active metabolite. All other strains of *Salmonella typhimurium* were not mutagenic in assays using PAPS.

Other *in vitro* Ames assays with estragole and metabolites of estragole have produced equivocal results. Estragole was very weakly positive without metabolic activation in TA100 and positive in TA100 with activation. No effect was seen in TA98. The 2,3-epoxide of estragole and 1'-hydroxyestragole were positive in strains TA100 and TA1535, but negative in

TA98 with or without S-13 metabolic activation [Swanson *et al.,* 1979]. But in a different study no evidence of mutagenicity was reported when 1'-hydroxyestragole was incubated with strains TA98 and TA100 of *Salmonella typhimurium* with and without S-13 metabolic activation. Addition of PAPS as a cofactor did not induce an increase in revertants. 1'-Acetoxyestragole was mutagenic in strains TA98 and TA100 but not in a dose-dependent manner [Drinkwater *et al.,* 1976]. Overall, estragole and its 1'hydroxymetabolite do not appear to be mutagenic in *Salmonella typhimurium*.

Estragole concentrations of 0.001 to 0.00001 M did not induce the formation of chromosomal aberrations in V79 cells with and without metabolic activation or in primary rat hepatocytes [Muller *et al.,* 1994].

In an unscheduled DNA synthesis (UDS) study, a marked increase in UDS was reported when primary rat hepatocytes were incubated with estragole concentrations of 0.001 to 0.00001 M [Muller *et al.*, 1994]. When freshly prepared hepatocytes from Fisher F344 male rats were incubated with concentrations of estragole in the range from 0.000001 to 0.01 M, a significant increase in UDS, as much as 2.7 times control values, occurred at concentrations in the range from 0.0001 to 0.01 M [Chan and Caldwell, 1992]. Cytotoxicity was observed at concentrations in the range from 0.0001 to 0.01 M. Incubation of the 1'-hydroxyestragole showed increased UDS at concentrations greater than 0.00001 to 0.000001 M. Lactate dehydrogenase (LDH) leakage occurred at greater than 0.0001 to 0.00001 M for 1'-hydroxyestragole. The UDS activity and cytotoxicity of estragole occurred at concentrations approximately an order of magnitude greater than those for the 1'-hydroxy metabolites. Additionally, cytotoxicity was observed at slightly higher concentrations than those needed to induce UDS, although the differences were minimal. A clear non-linear relationship and threshold were established between dose for estragole or 1'hydroxyestragole and UDS activity. Similar results were obtained for estragole in an earlier study [Howes *et al.,* 1990].

3.4.2.2 In vivo

Several *in vivo* genotoxicity assays are available for estragole. In an *in vivo* UDS study, hepatocytes isolated 4 or 12 hours after rats received a 500, 1000, or 2,000 mg/kg bw dose of estragole were evaluated for unscheduled DNA synthesis. Very slight increases in net grain counts were reported at the 500 and 1,000 mg/kg bw dose, but only at 2,000 mg/kg bw dose were the net grain counts greater than 5, which was the criteria for a positive result [Muller *et al*., 1994].

In a study designed to detect DNA adduct formation of estragole and the 1'-hydroxyestragole metabolite, adult female CD-1 mice (mean weight 35 g) were given 12 micromoles/mouse (58 mg/kg) of [2',3'-^{3}H]-1'-hydroxyestragole by intraperitoneal injection in trioctanoin and DNA adduct formation monitored over 20 days post exposure. Similarly, 9-day old male or female B6C3F1 mice (mean weight, 6g) were given intraperitoneal injections of 0.5 micromoles (14 mg/kg) of labeled estragole and sacrificed after 23 hours. Three adducts were formed by the reaction of 1' or 3' positions (*cis* or *trans* isomers) of estragole with the exocyclic amino group (N^2) of deoxyguanosine. An additional adduct was formed by the reaction of the 3' position of estragole and the (N^6) position of deoxyadenosine. Unlike adducts of aromatic amines (*e.g.*, N-acetyl-2-aminofluorene) which persist at near maximum levels of binding for several weeks, the three adducts of estragole-deoxyribonucleoside were removed rapidly from mouse liver DNA. Timed measurement of DNA adducts indicated a biphasic loss indicated by a sharp decline in one of the two major 1'-hydroxyestragole adducts followed by relatively constant levels of liver DNA adducts from days 3 to 20, suggesting excision repair [Phillips *et al.*, 1981].

In ^{32}P-post-labelling experiments with adult female CD-1 mice (mean weight, 25 g) a 2 or 10 mg dose of estragole was given by intraperitoneal injection and liver DNA samples were collected 24 hours later. The dose levels in this study were equivalent to 100 or 500 mg/kg bw, respectively. Estragole show binding activities higher than allylbenzene, anethole, and other allyl substituted benzene derivatives. A rapid drop in total adduct formation occurred within 7 days

after dosing and was followed by a relatively constant level over the next 140 days, an effect also observed in the previous study. The authors noted that the significant decrease in DNA adduct levels was probably related to DNA repair processes [Randerath *et al.*, 1984].

In a related ^{32}P-post-labelling experiment [Phillips *et al.*, 1984], newborn male B6C3F1 mice were given 0.25, 0.5, 1.0, and 3.0 micromoles of alkoxybenzene derivatives (including estragole, methyl eugenol and safrole) by intraperitoneal injection on day 1, 8, 15, and 22, respectively, after birth. Dose levels on days 1 and 22 were estimated to be approximately 27 and 35 mg/kg bw, 1'-hydroxyestragole and 1'-hydroxysafrole, respectively. Mice were terminated on days 23, 29, and 43 and their liver DNA was isolated and analyzed. Highest DNA adduct levels were measured for methyl eugenol, estragole, and safrole compared to controls or other substances tested. A significant (p less than 0.05) amount of adduct was detected at 43 days. Based on the results of a study of carcinogenic activity of these substances in the same species and strain (see Miller *et al.*, 1983 in Repeat Dose Toxicity], the authors concluded that adduct levels of at least 15 pmoles/mg of DNA at 23 days were required for statistically significant tumor formation [Phillips *et al.*, 1984]. The authors also noted that, compared to adults, newborn mice showed greater sensitivity to alkenylbenzene carcinogenicity.

3.4.2.3 Conclusions

The genotoxicity database on estragole shows no mutagenic potential in the Ames assay. In cytogenetic assays, there is no evidence of a genotoxic potential *in vitro*. *In vitro* UDS studies showed positive responses when rat hepatocytes were incubated with estragole. In an *in vivo* study, UDS was seen at the 2,000 mg/kg bw dose and very weak responses were seen at the 500 and 1,000 mg/kg bw doses. As demonstrated by the studies on DNA adduct formation, estragole forms DNA adducts when laboratory rodents are exposed to high dose levels, so it is not surprising that both substances and their 1'-hydroxy metabolites induce unscheduled DNA synthesis. In these studies, concentrations at which UDS occurs coincide with hepatocellular cytotoxicity. Based on the available data, no additional genotoxicity tests are recommended.

3.4.3 Repeat Dose Toxicity

Groups of CD-1 female mice (mean weight 24 g) were maintained on a diet containing 2,300 or 4,600 ppm estragole or 2,500 ppm 1'-hydroxy estragole for 10 months. The authors estimated that the dietary levels corresponded to an average daily intake of 150-300 and 300-600 mg/kg bw for animals on the 2,300 ppm and 4,600 ppm estragole diet, respectively, and 180-360 mg/kg bw for animals on the 1'-hydroxyestragole diet. To avoid intolerance the dietary concentration was reduced by 75% for the first 10 days and 50% for the next 10 days. The target diet was then maintained for 12 months. Survival at 20 months was slightly lower (68-70%) for estragole fed animals compared to control animals (78%). The average life span of mice given 1'-hydroxyestagole was 13.6 months compared to 18 months in controls. Body weights measured at 1, 4, and 8 months were markedly reduced at 4 and 8 months compared to controls. At 10 months, the incidence of hepatomas was 58% for animals at 2,300 ppm estragole, 71% for animals at 4,600 ppm estragole and 56% for animals at 2,500 ppm of 1'-hydroxyestragole and 0 % in controls. Histopathological examinations revealed portal fibrosis, chronic inflammation and bile duct proliferation in addition to the tumors. Varied number of ceroid-laden histocytes and focal area of hyperplasia and megalocytosis were also reported. Four mice fed 4,600 ppm estragole had hepatic angiosarcomas [Miller *et al.*, 1983].

Additionally, CD-1 mice (male (55) and female (49)) were administered 370 mg/kg of estragole by gavage twice a week for ten doses beginning at 4 days of age. The mice were weaned at 35 days of age. Hepatomas were observed as early as 11 months. At 14 months, 73% of the males (3.5 hepatomas/mouse) and 24% of control males (0.6 hepatomas/mouse) exhibited hepatomas. The incidence of hepatomas in females (9%, 0.1 hepatomas/mouse) was not statistically different from control females (2%, 0.02 hepatomas/mouse) [Miller *et al.*, 1983]. In another part of the study, male (50) and female (50) CD-1 mice were administered a total dose of 9.45 micromoles/mouse of estragole or estragole epoxide or 1.87 micromoles/mouse of 1'-hydroxyestragole by intraperitoneal injection distributed in a ratio of 1:2:4:8 on days 1, 8, 15, and 22, respectively, of life. These doses correspond to 0.63, 1.26, 2.52, and 5.04

micromoles/mouse, respectively. The mice were weaned at 22 days of age. At 12 months, 65% of the mice receiving estragole exhibited hepatomas (1.7 hepatomas/mouse) versus 26% of controls (0.5 hepatomas/mouse) exhibited hepatomas. The incidence of hepatomas in mice given estragole epoxide (40%, 0.6 hepatomas/mouse) was not statistically different from control (26%, 0.5 hepatomas/mouse). For 1'-hydroxyestragole, 93% of the mice receiving the test substance (2.7 hepatomas/mouse) and 15% of control males (0.2 hepatomas/mouse) exhibited hepatomas [Miller *et al.*, 1983]

In a study using a hybrid strain of B6C3F1 mice, and the parent strain, C3H/He male and female mice and C57BL/6 male and female mice, the mice were given intraperitoneal injections of 1'-hydroxyestragole on days 1, 8, 15, and 22. Dose levels were 0.1 micromoles on day 1, 0.04 micromoles on days 8 and 15, and 0.08 micromoles on day 22 after birth. The levels are calculated to provide 11.7 on day 1, 18.8 on day 8, 9.3 on day 15 and 10.1 mg/kg bw on day 22, respectively. The experiment was terminated after 14 months. The first tumor-bearing mouse was observed at 10 months. At 12 months, 76% of the treated C3H/He male mice (3.0 hepatomas/mouse) and 26% of control mice (0.3 hepatomas/mouse) exhibited hepatomas. The incidence of hepatomas in C3H/He female mice, 6% (0.06 hepatomas/mouse), was not statistically different from those of control females. For C57BL/6 mice, the incidence of hepatomas in treated males was 14% (0.3 hepatomas/mouse) and was 5% (0.07 hepatomas/mouse) in control males. No hepatomas were observed in treated or control B57BL/6 female mice [Wiseman *et al.*, 1987].

In another part of the study, groups of male B6C3F1 mice were given single intraperitoneal injections of 0.10 micromoles/g (15 mg/kg) bw of 1'-hydroxyestragole or 1'-hydroxysafrole 12 days after birth. Animals were sacrificed after 12 months and incidence of hepatic tumors were measured. A second group of males was given a lower dose of 0.01 micromoles/g bw. A statistically significant increase in the incidence of hepatomas/mouse were observed for both substances at 0.1 micromoles/g bw, but no significant increase was observed at the low dose of 0.01 micromoles/g bw (1.5 mg/kg) [Wiseman *et al.*, 1987].

In a NTP carcinogenesis bioassay, male and female F344/N rats and male and female B6C3F1 mice were administered methyl eugenol in 0.5% methylcellulose by gavage daily at dose levels of 37, 75, or 150 mg/kg bw per day, five days per week for 2 years [NTP, 2000]. Stop-exposure groups of rats received 300 mg/kg doses for 53 weeks followed by the vehicle only (0.5% methylcellulose) for the duration of the study. All rats at the highest dose level (150 mg/kg bw) and the stop-exposure dose level (300 mg/kg bw) died before the end of the study. Mean body weights of all dosed groups were less than those of the vehicle controls throughout the study. The incidences of liver non-neoplastic lesions in dosed groups of male and female rats were increased at 6 months, 12 months, and 2 years. There were statistically significant increases in oval cell hyperplasia, hepatocyte hypertrophy, and eosinophilic foci, at all dose levels in male and female rats. At the three highest doses (75, 150, and 300 mg/kg bw per day) atypical focal bile duct hyperplasia, focal cystic degeneration, and mixed cell foci were observed, more in males than females. Many of the same non-neoplastic lesions of the liver were reported in the 300 mg/kg bw groups of male and female rats at both 6 and 12 months in the stop-exposure group. Non-neoplastic lesions of the glandular stomach included statistically significant increases in mucosal atrophy at all dose levels and neuroendocrine hyperplasia at the three highest dose levels in females and at all dose levels in males. There was a significant increase in the incidence of nephropathy in females at 300 mg/kg, and the incidence of renal tubule hyperplasia was greater in the greater than or equal to 75 mg/kg groups than in the vehicle control.

Liver neoplasms related to methyl eugenol exposure were reported in all dose groups and included hepatocellular adenomas and carcinomas, hepatocholangiomas, and In hepatocholangiocarcinomas. In all treated male and female rat groups, statistically significant increases (P equal to 0.049 in males and P equal to 0.017 in females at 37 mg/kg bw; P less than 0.001 for all other treated groups) in the incidence of hepatocellular adenomas and carcinomas were reported. Hepatocholangiomas and hepatocholangiocarcinomas were reported in the 150 mg/kg bw group of males (2/50, 4%) and females (3/49, 6%) and at higher incidence in the 300 mg/kg bw stop-exposure groups of males (13/50, 26%) and females

(17/50, 34%). Both benign (3/50, 6%) and malignant (4/50, 8%) neuroendocrine cell neoplasms of the glandular stomach were reported in males at 150 mg/kg bw and in the 300 mg/kg bw stop-exposure group (2/49, 4.1% benign and 2/49, 4.1% malignant). The incidence of these neoplasms was much higher in females at dose levels of 75 mg/kg bw (13/50, 26% benign and 12/50, 24% malignant) and greater. In male rats, there were significant increases in the incidence of: malignant mesothelioma at 150 mg/kg; mammary gland fibroadenoma at 75 and 150 mg/kg; and fibroma of the subcutaneous tissue at 37 and 75 mg/kg. These neoplasms were not found in female rats at any dose level.

For mice, survival of all male dosed groups was similar to that of the vehicle controls. The survival of treated female mice was significantly less than those reported for control animals. Mean body weights of dosed mice were reported to be "generally less than those of the vehicle controls throughout the studies". In female mice and, to a lesser extent, in male mice there was evidence of hepatotoxicity of methyl eugenol. Significant increases in oval cell hyperplasia, eosinophilic foci, hepatocyte hypertrophy and necrosis, haematopoietic cell proliferation, haemosiderin pigmentation, and bile duct cysts were observed at all dose levels in male and female mice. Non-neoplastic lesions of the glandular stomach included statistically significant increases in hyperplasia, ectasia, atrophy at all dose levels in both males and females and mineralization and necrosis in lower incidence also in both sexes incidences of chronic atrophic gastritis was high. Gastric tumors were found in two high dose males. The incidence of hepatocellular adenomas, hepatocellular carcinomas and hepatoblastomas was high in both treated and control male and female mice. While control males and females showed tumor rates of 63% (31/49) and 50% (25/50), respectively, and all treatment groups of males and females had tumor rates in excess of 92% with the exception of high dose male rates in which the tumor rate was 82% (41/50). Evidence of infection by *Heliobacter hepaticus* was found by PCR-RFLP, but associated hepatitis was not found.

An extensive interpretation [Smith *et al*., 2002] of the NTP study concludes that the study was compromised by a number of factors including malnutrition in both species, toxicity at all dose

levels, gastric damage affecting the absorption, distribution, and metabolism of methyl eugenol, and the presence of infection in both sexes of mice. Also, the authors conclude that the study cannot be recognized as conclusive for carcinogenicity at lower, non-toxic dose levels of methyl eugenol. According to the authors (robust summary not included):

"The methyl eugenol bioassay was compromised by inappropriately high dose levels, administered by gavage, that cause significant hepatotoxicity, gastric damage, and malnutrition in both mice and rats. The presence of Heliobacter hepaticus in the livers of mice was also thought to have confounded the interpretation of the findings. Hepatic tumors occurred in severely damaged livers while the neuroendocrine tumors were likely to have resulted from endocrine responses to chronic gastric damage. At dose levels of methyl eugenol at which hepatic tumors occurred in rats, non-neoplastic liver changes such as liver and hepatocyte enlargement, necrosis, chronic inflammation, periportal fibrosis and nodular or adenomatoid hyperplasia, were invariably present. Such recurrent liver damage, in particular chronic inflammation and hyperplasia undoubtedly altered methyl eugenol metabolism and may have strongly enhanced the likelihood of DNA damage, fixation of relevant DNA damage and progression of initiated/pre-neoplastic cells to cancer. Therefore, the hepatotoxicity induced by high dose levels of methyl eugenol most probably plays a very significant, if not an essential, role in the formation of hepatic tumors. If in humans, exposure to high levels of methyl eugenol were to be accompanied by recurrent liver tissue damage and hyperplasia, methyl eugenol might possibly induce liver cancer in humans. However, if dose levels of methyl eugenol in humans are less than those needed to induce hepatotoxicity (most probably somewhere in the range of 1 to 10 mg/kg bw/day), exposure of humans to such non-hepatotoxic levels can be assumed to be associated with a very low, probably zero, cancer risk."

3.4.4 Reproductive Toxicity

Studies are available for a mixture of *p*-allylalkoxybenzene derivatives in three different species at multiple dose levels [Morgareidge, 1973a, 1973b, 1973c] and for the isomer methyl eugenol [Le Bourhis, 1973].

In an FDA sponsored study [Morgareidge, 1973a, 1973b, 1973c] that evaluated both reproductive and developmental toxicity parameters, the essential oil of nutmeg containing a mixture of *p*-allylalkoxybenzene derivatives {myristicin, safrole, elemicin, and methyl eugenol

(10-20%)} and bicyclic terpene $C_{10}H_{16}$ hydrocarbons {*alpha*-pinene, *beta*-pinene, and sabinene (80-90%)} was given to pregnant CD-1 mice, Wistar rats, or golden hamsters.

In the mouse study, groups (20-21/group) of pregnant female CD-1 outbred mice were given 0, 6, 26, 120, or 560 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil on days 6 through day 15 of gestation. A positive control group received 150 mg/kg bw per day of aspirin. Maternal body weights were recorded on days 0, 6, 11, 15, and 17 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On day 17 all dams were subjected to Caesarian section and the number of implantation sites, resorption sites, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, number of implantation sites, number of corpora lutea, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported. The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10x magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects.

The administration of up to and including 560 mg/kg bw per day of test article FDA 71-28 to pregnant mice on days 6 through 15 of gestation had no effects on nidation, reproduction, maternal survival or any measured fetal parameter. The number and types of abnormalities seen in tissues of the dam or pups of the test groups did not differ for the number and type occurring spontaneously in the positive or negative controls.

The rat and hamster studies use the same study protocol as that used for the mouse study. Adult female Wistar or golden hamsters were individually housed in mess-bottom cages in a temperature- and humidity-controlled room. They were mated with untreated young adult males and observation of vaginal sperm plugs (rats) or appearance of motile sperm in vaginal smears

(hamsters) was considered day 0 of gestation. Groups (22-23/dose) of pregnant Wistar rats were then given 0, 3, 2, 56, or 260 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil daily on day 6 and through day 15 of gestation [Morgareidge, 1973c]. Groups (26-28/dose) of pregnant hamsters were given 0, 6, 28, 130, or 600 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil daily on day 6 and through day 10 of gestation [Morgareidge, 1973b]. In the rats or hamster study, a positive control group received 250 mg/kg bw per day of aspirin.

The administration of up to and including 260 mg/kg bw per day of test article FDA 71-28 to pregnant rats on days 6 through 15 of gestation or administration of up to and including 600 mg/kg bw per day to pregnant golden hamsters on day 6 through day 10 of gestation had no effects on nidation, reproduction, maternal survival or any measured fetal parameter.

In the three-species study, no reproductive effects were observed when daily dose levels of up to 260 to 600 mg/kg bw of the essential oil predominantly composed of a combination of *p*-allylalkoxybenzene derivatives (10-20%) and bicyclic terpene hydrocarbons was administered daily to mice, rats, or hamsters during gestation. These dose levels correspond to dose levels of 26 to 120 mg/kg bw per day of *p*-allylalkoxybenzene derivatives. When this data is combined with the fact that no adverse effects were observed to the reproductive organs in 4-generation study with the double bond isomer anethole (see below), it is concluded that *p*-allylalkoxybenzene derivatives exhibit a low potential to produce reproductive toxicity.

In a comprehensive 4-generation study, groups of male and female rats (F_0) were fed 0 or 1% anethole in the diet (approximately 600-1,500 mg/kg bw per day) prior to mating, during the 15-day mating period, and during gestation and lactation. Offspring (F_1) were used for propagating the next generation and were raised on the same dietary treatment as their parents. A similar procedure was followed to obtain the 3rd and 4th generations (F_2 and F_3). The only notable effect was reduced body weight gain and body weights coinciding with reduced feed

intake in rats fed 1% anethole. There was no effect on reproductive performance over 4 generations. The reduced palatability of the diet was considered to be responsible for the lower body weight gain and body weights of the rats receiving anethole.

To ascertain the effect of palatability on the effects reported in the 4-generation study, a cross-fostering experiment was conducted using groups of control and treated F_1 females (from the 4-generation study and receiving 1% anethole in the diet) mated with control F_1 males (from the 4-generation study) [Le Bourhis, 1973]. Litters born from treated females were exchanged with litters from control females at birth and reared by the new dams. No significant difference in body weights of pups from those nursed by mothers of the same group, regardless from which group they were born, was reported and final body weights of pups born from treated dams but raised by control dams regained normal values by day 28. The results indicated that postnatal growth is not directly affected by anethole exposure, but is a result of the nutritional status of the dams [Le Bourhis, 1973].

Based on the results of reproductive toxicity on an essential oil containing a mixture of *p*-allylalkoxybenzene derivatives and an isomer anethole, no further testing on the possible reproductive toxicity of estragole is recommended.

3.4.5 Teratogenicity/Developmental Toxicity

A developmental study is available for the structurally related substance 4-methoxy-1-propenylbenzene (*trans*-anethole). In a developmental and reproductive screening test, groups of female rats were administered 0, 35, 175, or 350 mg anethole/kg bw per day *via* gavage in corn oil for 7 days prior to co-habitation with male rats until day 4 of lactation. The only notable effects were reduced mean body weights and decreased feed consumption in high-dose rats. These effects were seen to some extent in rats gavaged with anethole 175 mg/kg bw per day, but only reached statistical significance in the early part of the study. At the high dose (350 mg/kg bw per day), the number of liveborn pups was significantly decreased, the number of

stillborn pups was significantly increased, the number of pups dying on day 1 and days 2-4 was significantly increased, the viability index (number of live pups on postpartum day 4/number of liveborn pups on postpartum day 1) was significantly decreased, the number of surviving pups/litter on postpartum day 4 was significantly decreased, the live litter size on postpartum day 4 was significantly decreased, and pup weight/litter on postpartum day 1 was significantly decreased compared to controls. No anomalies and no other effects were reported. The authors determined the maternal and developmental no observable adverse effect level (NOAEL) to be 35 and 175 mg/kg bw per day, respectively, and the maternal and developmental lowest observable adverse effect level (LOAEL) to be 175 and 350 mg/kg bw per day, respectively. Anethole did not cause any effects on the rat fetus at doses below those causing maternal toxicity (reduced body weight and feed consumption).

In the FDA sponsored study discussed above [Morgareidge, 1973a, 1973b, 1973c], female pregnant CD-1 mice, Wistar rats, and golden hamsters were given dose levels of up 560, 260, and 600 mg/kg bw, respectively, of an essential oil containing 10-20% *p*-allylalkoxybenzene derivatives and 80-90% bicyclic terpene hydrocarbons daily by gavage during gestation. Based on clinical observations and measurement of body weight gain, mortality, and evaluation of the urogenital tract of pregnant females there were no signs of maternal toxicity at any dose level in any of the three species. Based on measurements of fetal survival, fetal body weight, visceral examination of pups, and a complete skeletal examination of pups at all dose levels, there was no evidence of developmental toxicity at any dose level in any of the three species.

Additionally, a developmental study is available for the related substance, safrole. No teratogenic effects were reported when safrole was administered intragastrically to female Swiss mice from days 6-14 of pregnancy [Moro *et al*., 1985].

Based on the lack of maternal and developmental toxicity in a four-generation study with the alkene isomer anethole, a developmental study with safrole, and a three-species study at

multiple dose levels of an essential oil containing a mixture containing *p*-allylalkoxybenzene derivatives [Morgareidge, 1973a, 1973b, 1973c], it is concluded that estragole is not a maternal or developmental toxicant.

No additional testing is recommended given the available data.

3.4.6 New Testing Required

None.

3.5 TEST PLAN TABLE

Chemical	Physical-Chemical Properties				
	Melting Point	**Boiling Point**	**Vapor Pressure**	**Partition Coefficient**	**Water Solubility**
Estragole CAS No. 140-67-0	Calc	A	A	Calc	A

Chemical	Environmental Fate and Pathways			
	Photodegradation	**Stability in Water**	**Biodegradation**	**Fugacity**
Estragole CAS No. 140-67-0	Calc	NA	R, Test	Calc

Chemical	Ecotoxicity		
	Acute Toxicity to Fish	**Acute Toxicity to Aquatic Invertebrates**	**Acute Toxicity to Aquatic Plants**
Estragole CAS No. 140-67-0	R, Test	R	R, Test

Chemical	Human Health Data					
	Acute Toxicity	**Genetic Toxicity *In Vitro***	**Genetic Toxicity *In Vivo***	**Repeat Dose Toxicity**	**Repro-ductive Toxicity**	**Develop-mental Toxicity**
Estragole CAS No. 140-67-0	A	A	A	A	R	R

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using category approach, SAR
Test	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties
O	Other

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AR201-14022B

The Flavor And Fragrance High Production Volume Consortia

The Terpene Consortium

Robust Summaries for Estragole

Estragole **CAS No. 140-67-0**

FFHPVC Terpene Consortium Registration Number

Submitted to the EPA under the HPV Challenge Program by:
The Flavor and Fragrance High Production Volume Chemical Consortia
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Robust Summaries for Estragole

The evaluation of the quality of the following data uses a systematic approach described by Klimisch [Klimisch *et al*., 1996]. Based on criteria relating to international testing standards for categorizing data reliability, four reliability categories have been established. The following categories are:

- Reliability code 1. Reliable without restrictions
- Reliability code 2. Reliable with restrictions
- Reliability code 3. Not reliable
- Reliability code 4. Not assignable

1 CHEMICAL AND PHYSICAL PROPERTIES

1.1 Melting Point

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	Calculated/Mean or weighted (adapted Stein and Brown method)
GLP	No
Melting Point	-1.19 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVP EPI Suite (2000) U S Environmental Protection Agency.

1.2 Boiling Point

Substance Name	Estragole
CAS No.	140-67-0
GLP	Ambiguous
Boiling Point	216 deg C
Pressure	764
Pressure Unit	mm Hg
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Merck Index (1998) The Merck Index, 12th edition, Merck & Co., Inc. Whitehouse Station, NJ.

Substance Name	Estragole
CAS No.	140-67-0
GLP	Ambiguous
Boiling Point	216 °C
Pressure	760
Pressure Unit	mm Hg
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Fragrance Materials Association (FMA) Reported values for boiling point of estragole.

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	Adapted Stein and Brown method
GLP	No
Boiling Point	209.93 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.

Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVP EPI Suite (2000) U S Environmental Protection Agency.

1.3 Vapor Pressure

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	Experimental
GLP	No
Year	1947
Vapor Pressure	1 mm Hg
Temperature	52.6 °C
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Stull D.R. (1947) Vapor pressure of pure substances. Organic Compounds. Ind Eng Chem., 39, 517-540.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for anethole, isomer unspecified
Method/guideline	Measured
GLP	Ambiguous
Vapor Pressure	0.041 mm Hg (5.45 Pa)
Temperature	21 °C (294 K)
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Daubert T.E. and Danner, R.P. (1989) Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Taylor and Francis, Washington, DC.418

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	Calculated
Vapor Pressure	0.09 mm Hg (12 Pa)
Temperature	20 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Fragrance Materials Association (FMA) Reported values of vapor pressure for estragole. Unpublished report.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for *trans*-anethole
Method/guideline	Calculated
Vapor Pressure	0.05 mm Hg (6.67 Pa)
Temperature	20 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Fragrance Materials Association (FMA) Reported values of vapor pressure for trans-anethole. Unpublished report.

1.4 n-Octanol/Water Partition Coefficients

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	Calculated
Log Pow	3.47
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.

References	KOWWIN EPI Suite (2000) U.S. Environmental Protection Agency.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for *trans*-anethole
Method/guideline	Calculated
Log Pow	3.39
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	KOWWIN EPI Suite (2000) U.S. Environmental Protection Agency.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for *trans*-anethole
Method/guideline	Calculated
Log Pow	3.11
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Interactive Analysis LogP and LogW Predictor: Database contributed by Syracuse Research Corporation, SciVision, Albany Molecular Research, Inc., eduSoft LC, Cambridge Soft. www.logp.com.

1.5 Water Solubility

Substance Name	Estragole
CAS No.	140-67-0
Method/Guideline	Measured
GLP	Ambiguous

Year	1992
Value (mg/L) at Temperature	178 mg/L at 25 °C
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	WSKOWIN EPI Suite (2000a) U S Environmental Protection Agency (Yalkowski, S.H. and Dannenfelser, R.M., 1992)

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for anethole, isomer unspecified
Method/Guideline	Measured
GLP	No
Value (mg/L) at Temperature	111 mg/L at 25 °C
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: peer reviewed reference
References	WSKOW EPI Suite (2000a) U S Environmental Protection Agency (Yalkowski S.H., and Dannenfelser, R.M., 1992)

Substance Name	Estragole
CAS No.	140-67-0
Method/Guideline	Calculated
Remarks for Test Conditions	Used an estimated log Kow of 3.47
Value (mg/L) at Temperature	84.55 mg/L at 25 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	WSKOWIN EPI Suite (2000b) US Environmental Protection Agency.

2 ENVIRONMENTAL FATE AND PATHWAYS

2.1 Photodegradation

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	Calculated
Test Type	AOPWIN
Halflife t1/2	2.36 hours
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	AOPWIN EPI Suite (2000) US Environmental Protection Agency.

2.2 Biodegradation

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for *p*-(2-propenyl)anisole isomer, anethole
Method	OECD Guideline 301B
Test Type	Sealed vessel test (CO2 production test)
Year	1994
Innoculum	10% by volume of secondary effluent from an unacclimatized activated sludge
Remarks for Test Conditions	The test concentration was nominal 10 mg/L organic carbon with a test temperature range of 20-24 °C. The mean percentage biodegradation was calculated from 4 vessels on day 28.
Degradation % After Time	91.0% (90.7-91.2%)
10 day window criteria	Yes

Total degradation	Yes
Conclusion Remarks	Anethole is classified as readily and ultimately biodegradable.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
Reference	Quest International, Inc. (1994) The ultimate and readily biodegradation of anethole. Unpublished report.

Substance Name	Estragole
CAS No.	140-67-0
Method	Calculated
Test Type	BIOWIN
Results	Probability of rapid biodegradation - linear model 0.8636 - nonlinear 0.9766. Expert survey results - Ultimate survey model: 2.7387 (weeks-months); Primary survey model: 3.6425 (days-weeks)
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
Reference	BIOWIN EPI Suite (2000) U S Environmental Protection Agency (Meylan W., 1994).

2.3 Fugacity

Substance Name	Estragole
CAS No.	140-67-0
Model Conditions	25 °C, 100,000 pounds
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	Level III
Input Parameters	MW, log Kow, water solubility, calculated MP & VP
Media	Air
Estimated Distribution and Media Concentration	0.556%

Remarks	Half-life = 3.92 hours
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	The data are obtained by a recognized fugacity calculation method. Data are considered reliable with restriction because this method does not allow for biodegradation or metabolism.
References	Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a, 1996b) Assessing the fate of new and existing chemicals: a five-stage process & Evaluating the fate of a variety of types of chemicals using the EQC model. Env. Tox.& Chem., 15(9), 1618-1637.

Substance Name	Estragole
CAS No.	140-67-0
Model Conditions	25 °C, 100,000 pounds
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	Level III
Input Parameters	MW, log Kow, water solubility, calculated MP & VP
Media	Water
Estimated Distribution and Media Concentration	19.7%
Remarks	Half-life = 900 hours
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	The data are obtained by a recognized fugacity calculation method. Data are considered reliable with restriction because this method does not allow for biodegradation or metabolism.
References	Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a, 1996b) Assessing the fate of new and existing chemicals: a five-stage process & Evaluating the fate of a variety of types of chemicals using the EQC model. Env. Tox.& Chem., 15(9), 1618-1637.

Substance Name	Estragole
CAS No.	140-67-0
Model Conditions	25 °C, 100,000 pounds
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay

Model Used	Level III
Input Parameters	MW, log Kow, water solubility, calculated MP & VP
Media	Soil
Estimated Distribution and Media Concentration	78.8%
Remarks	Half-life = 900 hours
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	The data are obtained by a recognized fugacity calculation method. Data are considered reliable with restriction because this method does not allow for biodegradation or metabolism.
References	Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a, 1996b) Assessing the fate of new and existing chemicals: a five-stage process & Evaluating the fate of a variety of types of chemicals using the EQC model. Env. Tox.& Chem., 15(9), 1618-1637.

Substance Name	Estragole
CAS No.	140-67-0
Model Conditions	25 °C, 100,000 pounds
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	Level III
Input Parameters	MW, log Kow, water solubility, calculated MP & VP
Media	Sediment
Estimated Distribution and Media Concentration	0.88%
Remarks	Half-life = 3600 hours
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	The data are obtained by a recognized fugacity calculation method. Data are considered reliable with restriction because this method does not allow for biodegradation or metabolism.
References	Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a, 1996b) Assessing the fate of new and existing chemicals: a five-stage process & Evaluating the fate of a variety of types of chemicals using the EQC model. Env. Tox.& Chem., 15(9), 1618-1637.

3 ECOTOXICITY

3.1 Acute Toxicity to Fish

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for *trans*-anethole, purity greater than 99%
Method/guideline	96-hour LC50 continuous flow (ASTM, 1989)
Test Type	Experimental
GLP	Ambiguous
Year	1989
Species/Strain/Supplier	Minnows/Flathead
Exposure Period	96 hour
Analytical monitoring	GC Analysis
Remarks for Test Conditions	Temperature = 24.8 °C, dissolved oxygen = 6.4 mg/L, hardness = 39.4 mg/L CaCO3, alkalinity 30.6 mg/L CaCO3, tank volume = 1 L, pH = 7.6 Fish sizes: mean length=16.7 mm; mean weight=0.07 mm; loading 1.4 g/L; age=30 days Stock solutions (49 mg/L) were prepared daily and supplied to the proportional diluter.
Observations of Precipitation	None
Endpoint value	LC50 = 7.690 mg/L; EC50 = 4.810 mg/L
Nominal concentrations as mg/L	0.9, 16, 8, and 25.8 mg/L
Measured concentrations as mg/L	Corrected average: Less than 0.06, 2.73, 3.96, 5.85, 10.1, and 17.
Remarks fields for results	Confidence limits could not be reliably calculated. Test tanks were not sampled at 96 hours. Volatility caused actual concentrations to be less than nominal.
Unit	mg/L
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.

Reference	Broderius S., Hammermeister, D., Russom, C. (1990) Toxicity of eight terpenes to fathead minnows (*Pimephales promelas*), daphnids (*Daphnia magna*), and algae (*Selanastrum capriucornutum*). US EPA Environmental Research Laboratory/AScI Corporation. Unpublished. American Society of Testing and Materials (ASTM) 1989. Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. E729. In: Vol. 11.04 of 1989 Annual Book of ASTM Standards, pp. 336-355.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for methyl eugenol
Test Type	Experimental
GLP	No
Year	1975
Species/Strain/Supplier	Fish/Rainbow trout
Exposure Period	96 hour
Remarks for Test Conditions	Ten fish were used. Each material tested at 5 concentrations. Control groups conducted concurrently. The fish were observed for 96 hours.
Nominal concentrations as mg/L	3.2-10 mg/L
Endpoint value	6 mg/L 95% C.I. (4.9-7.2)
Reference substances (if used)	Toxaphene
Conclusion remarks	The authors concluded that estragole was of a low order of toxicity to fish.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
Reference	Beroza M., Inscoe M., Schwartz P., Kepliknger M. and Mastri C. (1975) Toxicology and Applied Pharmacology 31, 421-429.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for methyl eugenol
Test Type	Experimental

GLP	No
Year	1975
Species/Strain/Supplier	Fish/Bluegill sunfish
Exposure Period	96 hour
Remarks for Test Conditions	Ten fish were used. Each material tested at 5 concentrations. Control groups conducted concurrently. The fish were observed for 96 hours.
Nominal concentrations as mg/L	3.2-10 mg/L
Endpoint value	8.1 mg/L 95% C.I. (7.4-9.0)
Reference substances (if used)	Toxaphene
Conclusion remarks	The authors concluded that estragole was of a low order of toxicity to fish.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
Reference	Beroza M., Inscoe M., Schwartz P., Kepliknger M. and Mastri C. (1975) Toxicology and Applied Pharmacology 31, 421-429.

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	ECOSAR
Test Type	Calculated
Species/Strain/Supplier	Fish
Exposure Period	96 hours
Remarks for Test Conditions	Based on: log KOW = 3.47 and water solubility = 178 mg/L at 25 °C.
Endpoint value	LC50 = 4.561 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U S Environmental Protection Agency.

3.2 Acute Toxicity to Aquatic Invertebrates

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Test substance was estragon oil (tarragon oil). Typical composition of estragon oil is (70-88% estragole).
Method/guideline	OECD Guideline 202-I
Test Type	Experimental
GLP	Yes
Year	2001
Species/Strain/Supplier	*Daphnia magna/Straus*
Test Details	48 hours
Remarks for Test Conditions	Groups of 20 Daphnia magna (Karlsruhe, GDR)(5/1ml test volume) were exposed to test concentrations of 0, 0 (acetone solvent), 3.8,7.5,15.0, 30.0, or 60.0 mg/L of estragon oil for 48 hours. Solution temperature and pH were maintained at 20-20.5 C and 7.98. Invertebrates were held for 16 hours in daylight followed by 8 hours of dark. The conductivity of the water was 0.4 to 1.5 uS/cm and water hardness was 200 mg/L.
Nominal concentrations as mg/L	0,3.8,7.5,15.0, 30.0, or 60.0
Unit	mg/L
EC50, EL50, LC0, at 24,48 hours	EC50 = 30.5mg/l (95% CI, 13.3-48 mg/L)
Biological observations	No reduction in swimming mobility was observed at 0, 3.8, 7.5 or 15 mg/L at 3, 24, or 48 hours. At 30.0 mg/L reduction in swimming mobility was reported for 5/20, 5/20, 8/20 at 3, 24, or 48 hours, respectively.
Control response satisfactory?	Yes
Appropriate statistical evaluations?	Probit Analysis
Remarks fields for results	Measurement of pH, Oxygen concentration, and temperature at 0 and 48 hours revealed no significant change (7.69-8.02) in pH, O2 concentration (8.3-8.6), or temperature (20 to 20.3C)
Conclusion remarks	The EC50 for *Daphnia magna* in a static immobilization study was 30.5 mg/L
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Data Reliability Remarks	Code 1. Guideline study.

Reference	Barth M. and Winkler, J (2001) Testing for acute toxicity of estragon oil (*Artemisia dracunculus L.)* in Daphne - *Daphnia magna*. Unpublished report.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data for *p*-(2-propenyl)anisole isomer, (*trans*-anethole, Purity greater than 99%
Method/guideline	48-hr LC50 continuous flow (ASTM, 1989)
Test Type	Experimental
GLP	Ambiguous
Year	1990
Analytical procedures	GLC Analysis
Species/ Strain/Supplier	*Daphnia magna*
Test Details	48 hours
Remarks for Test Conditions	Temperature =1 9.7 °C, dissolved oxygen = 7.8 mg/L, hardness = 45.5 mg/L CaCO3, alkalinity 36.8 mg/L CaCO3, tank volume = 0.20 L, pH = 8.0 *Daphnid agie* less than 24 hours, Stock solution = 15.2 mg/L
Nominal concentrations as mg/L	0, 3.04, 6.08, 9.12, 12.2
Measured concentrations as mg/L	Corrected average: Less than 0.06, 2.84, 5.42, 7.13, 10.9, and 14.5 mg/L
Unit	mg/L
EC50, EL50, LC0, at 24,48 hours	48-hour LC50 = 6.82 mg/L
Appropriate statistical evaluations?	Yes
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Data Reliability Remarks	Code 1. Comparable to guideline study.
Reference	Broderius S., Hammermeister D., Russom, C. (1990) Toxicity of eight terpenes to flathead minnows (*Pimephales promelas*), Daphnids (*Daphia magna*), and algae (*Selanastrum capriucornutum*.) US EPA Environmental Res. Lab./ASci Corp. Unpublished report.

Substance Name	Estragole
CAS No.	140-67-0

Method/guideline	ECOSAR
Test Type	Calculated
Species/Strain/Supplier	*Daphnia magna*
Test Details	48 hours
Remarks for Test Conditions	Based on: log KOW = 3.47 and water solubility = 178 mg/L at 25 C.
Unit	mg/L
EC50, EL50, LC0, at 24,48 hours	LC50 = 5.410 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Data Reliability Remarks	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U S Environmental Protection Agency.

3.3 Acute Toxicity to Aquatic Plants

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data for *trans*-anethole, Purity greater than 99%
Method/guideline	Static 96-hour toxicity test (ASTM, 1988)
Test Type	Experimental
GLP	Ambiguous
Year	1990
Species/Strain/Supplier	Green algae
Exposure Period	72 to 96 hours
Remarks for Test Conditions	Because of volatility issues, 75 mL of test solution were placed in 125 mL flasks to minimize headspace. Five concentrations of stock were tested: 100, 50, 25, 12.5, and 0% in replicates of 4 and shaken continuously. Test cell concentrations were about 1x10E4 cell/mL. IC50 was calculated using a linear interpolation program (Marcus and Holtzman, 1988; Norberg-King, 1988)
Endpoint Value	96-hour IC50 = 9.571 mg/L (CI:7.434-13.274)

Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
Reference	Broderius S., Hammermeister, D., Russom, C. (1990) Toxicity of eight terpenes to fathead minnows (*Pimephales promelas*), daphnids (*Daphnia magna*), and algae (*Selanastrum capriucornutum*). US EPA Environmental Res Lab/AScI Corporation. Unpublished report.

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	ECOSAR
Test Type	Calculated
Species/Strain/Supplier	Green algae
Exposure Period	96 hour
Remarks for Test Conditions	Based on: log KOW = 3.47 and water solubility = 178 mg/L at 25 °C.
Endpoint Value	EC50 = 3.681mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U S Environmental Protection Agency.

4 HUMAN HEALTH TOXICITY

4.1 Acute Toxicity

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	Litchfield and Wilcoxon, 1949
Test Type	Oral LD50
GLP	No
Year	1964
Species/strain	Rat/Osborne Mendel
Sex	Male and Female
# of animals per sex per dose	5
Vehicle	None
Route of Administration	Oral-Gavage
Remarks for Test Conditions	The test material was administered to 5 male and 5 female Osborne-Mendel rats per dose. Animals were fasted for 18 hours prior to dosing. All doses were given by intubation. Observations for two weeks included mortality and/or systemic effects. LD50 results were calculated using Litchfield-Wilcoxon (1949).
Value LD50 or LC50 with confidence limits	1820 mg/kg bw 95% confidence limits = 1670-1980 mg/kg bw.
Number of deaths at each dose level	Not given
Remarks for Results	Death from 4 hours to 8 days. Toxic signs included depression, coma, rough fur, wet posterior and porpyrin-like deposits around eye reported as toxic sign.
Conclusion remarks	The oral LD50 was calculated to be1820 mg/kg bw with 95% confidence limits = 1670-1980 mg/kg bw.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Jenner P.M., Hagan E.C., Taylor J.M., Cook E.L. and Fitzhugh O.G. (1964) Food flavorings and compounds of related structure I. Acute oral toxicity. Food and Cosmetics Toxicology, 2(3), 327-343.

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	Not given
Test Type	Oral LD50
GLP	No
Year	1972
Species/strain	Rabbit/New Zealand White
Sex	Not reported
Number of animals per sex per dose	10
Vehicle	None
Route of Administration	Dermal
Remarks for Test Conditions	Ten New Zealand white rabbits were administered the test substance on their clipped abraded abdominal skin. Observations made for mortality and toxic effects.
Value LD50 or LC50 with confidence limits	Greater than 5000 mg/kg bw
Number of deaths at each dose level	0/10 deaths
Conclusion Remarks	The dermal LD50 was reported to be greater than 5000 mg/kg bw.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Moreno O. (1972a) Acute dermal toxicity of estragole in rabbits. Unpublished report to RIFM.

Substance Name	Estragole
CAS No.	140-67-0
Method/Guideline	Litchfield and Wilcoxon, 1949
Test Type	Oral LD50
GLP	No
Year	1964
Species/strain	Mouse

Sex	Not reported
Vehicle	None
Route of Administration	Oral-Gavage
Remarks for Test Conditions	Oral doses of test substance given to mice on full stomachs. Doses administered *via* intubation. Mice observed for two weeks.
Value LD50 or LC50 with confidence limits	1250 mg/kg bw 95% confidence limits = 812-1920 mg/kg bw
Number of deaths at each dose level	Not given
Remarks for Results	Death from 1 hour to 4 days. Toxic signs included depression and coma at higher doses.
Conclusion Remarks	The oral LD50 was calculated to be1250 mg/kg bw with 95% confidence limits = 812-1920 mg/kg bw.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Jenner P.M., Hagan E.C., Taylor J.M., Cook E.L. and Fitzhugh O.G. (1964) Food flavorings and compounds of related structure I. Acute oral toxicity. Food and Cosmetics Toxicology, 2(3), 327-343.

Substance Name	Estragole
CAS No.	140-67-0
Method/Guideline	Not given
Test Type	Oral LD50
GLP	No
Year	1972
Species/strain	Rat/Wistar
Sex	Male
Number of animals per sex per dose	10
Vehicle	None
Route of Administration	Oral
Remarks for Test Conditions	Ten male albino Wistar rats per group were used. Animals were fasted for a minimum of 16 hours prior to administration of the test material. Animals weighed 200-250 grams. Following dosing the animals received food and water *ad libitum*. Observations for mortality were made at 1 and 6 hours after dosing and daily thereafter for 14 days. Toxic effects were also observed. Gross necropsies were performed on all survivors.

	observed. Gross necropsies were performed on all survivors.
Value LD50 or LC50 with confidence limits	1230 mg/kg bw 95% Confidence Limits (1080-1380 mg/kg bw)
Number of deaths at each dose level	820 mg/kg bw: No observable effects, 1030 mg/kg bw: 2/10 deaths, 1230 mg/kg bw: LD50, 1280 mg/kg bw: 6/10 deaths; 1600 mg/kg bw 9/10 deaths.
Conclusion Remarks	The oral LD50 was calculated to be 1230 mg/kg bw with confidence limits of 1080-1380 mg/kg bw.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Moreno O. (1972b) Acute oral toxicity of estragole in rats. Unpublished report to RIFM.

4.2 Genetic Toxicity

4.2.1 *In vitro* Genotoxicity

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Purity 99.9%
Method/guideline	Ames
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	Ambiguous
Year	1982
Species/Strain	*Salmonella typhimurium* TA 98, TA 100, TA 1535, and TA 1537
Metabolic Activation	With and without rat liver microsome fraction S9 from Aroclor induced rats
Doses/Concentration	30-300 micrograms/plate
Statistical Methods	Student's t test
Remarks for Test Conditions	The assays with S9 were conducted using the pre-incubation method, while the assays without S-9 were conducted using the plate incorporation method.

Results	Negative
Cytotoxic concentration	Not given
Genotoxic Effects	None
Appropriate statistical evaluations?	Yes
Remarks for Results	Estragole was inactive in *Salmonella* strains TA 1535, TA 1537, TA 98 & TA 100 both in the presence and absence of metabolic activation.
Conclusion Remarks	No evidence of mutagenicity.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Sekizawa J. and Shibamoto T. (1982) Genotoxicity of safrole-related chemicals in microbial test systems. Mutation Research. 101(1), 127-140.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Purity 99.9%
Method/guideline	Ames
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	Ambiguous
Year	1982
Species/Strain	*Escherichia coli* WP2 uvrA trp-
Metabolic Activation	With and without rat liver microsome fraction S9 from Aroclor induced rats
Doses/Concentration	30-300 micrograms/plate
Statistical Methods	Student's t test
Remarks for Test Conditions	Conducted as in Ames except that histidine was replaced with tryptophan
Results	Negative
Cytotoxic concentration	Not given
Genotoxic Effects	None
Appropriate statistical evaluations?	Yes

Remarks for Results	Estragole was inactive in *E. coli* WPR uvrA both in the presence and absence of metabolic activation.
Conclusion Remarks	No evidence of mutagenicity.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Sekizawa J. and Shibamoto T. (1982) Genotoxicity of safrole-related chemicals in microbial test systems. Mutation Research. 101(1), 127-140.

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	Ames
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	No
Year	1977
Species/Strain	*Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537, and TA1538
Metabolic Activation	None
Doses/Concentration	0.2 micromolar or 30 micrograms (calculated based on MW of 148.21)
Statistical Methods	Not given
Remarks for Test Conditions	The solvent used was ethanol.
Results	Negative
Cytotoxic concentration	Not given
Genotoxic Effects	None
Appropriate statistical evaluations?	None given
Remarks for Results	Negative
Conclusion Remarks	No evidence of mutagenicity.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Dorange J. L., Delaforge M. Janiaud P. and Padieu P. (1977) Mutagenicity of the metabolites of the epoxide diol pathway of safrole and analogs. Study on *Salmonella typhimurium*. Societe de Biologie de Dijon, 171(5), 1041-1048.

de Biologie de Dijon, 171(5), 1041-1048.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Purity 99.9%
Method/guideline	Ames
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	Ambiguous
Year	1991
Species/Strain	*Salmonella typhimurium* TA 98, TA 100, TA 1535 and TA 1537
Metabolic Activation	With and without rat liver microsome fraction S9 from Aroclor induced rats
Doses/Concentration	0.06-0.5 microliters/plate (0.06-0.48 micrograms/plate)
Statistical Methods	Not given
Remarks for Test Conditions	The solvent used was DMSO. The pre-incubation method was used.
Results	Negative
Cytotoxic concentration	Not given
Genotoxic Effects	None
Appropriate statistical evaluations?	None given
Remarks for results	Negative
Conclusion Remarks	No evidence of mutagenic activity.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Zani F., Massimo G., Benvenuti S., Bianchi A., Albasini A., Melegari M., Vampa G., Bellotti A., Mazza P. (1991) Studies on the genotoxic properties of essential oils with Bacillus subtilis rec-assay and Salmonella microsome reversion assay. Planta Medica, 57(3), 237-241.

Substance Name	Estragole
CAS No.	140-67-0

Method/guideline	Ames
Test Type	Ames reverse mutation
System of Testing	Bacterial
GLP	Ambiguous
Year	1987
Species/Strain	*Salmonella typhimurium* TA 97, TA 98, TA 100, TA 1535, and TA 1537
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced rats
Doses/Concentration	1-200 micrograms/ml
Statistical Methods	Not given
Remarks for Test Conditions	The pre-incubation method was used. The vehicle was DMSO.
Results	Negative
Cytotoxic concentration	Not given
Genotoxic Effects	None
Appropriate statistical evaluations?	None given
Remarks for results	Estragole was inactive in *Salmonella* strains TA 1535, TA 1537, TA 97, TA 98 & TA 100 both in the presence and absence of metabolic activation system.
Conclusion Remarks	No evidence of mutagenicity.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Zeiger E, Anderson B., Haworth S. Lawlor T., Mortelmans K. and Speck W. (1987) Salmonella mutagenicity tests: III. Results from testing 255 chemicals. Environmental Mutagenesis 9(9), 1-109.

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	Ames
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	Ambiguous
Year	1982

Species/Strain	*Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537, and TA1538
Metabolic Activation	With and without rat liver microsome fraction S9 from Aroclor induced rats
Doses/Concentration	0.05 -50 micrograms/plate
Statistical Methods	Not given
Remarks for Test Conditions	An Ames plate incorporation test was conducted with and without metabolic activation in strains TA1535, TA100, TA1537, TA1538 and TA98. The vehicle and negative control was ethanol. Metabolic activation was provided by liver S9 prepared from Aroclor 1254-induced rats. The positive control was 10.0 ug/plate 2-aminoanthracine. For strain TA1538, metabolic activation was provided by 3'-phosphoadenosine-5'-phosphosulfate (PAPS) and with and without liver S9 prepared from Aroclor 1254-induced rats.
Results	No mutagenic effects except a significant increase in the revertants per plate was reported for strain TA1538 in the presence of S-9 and PAPS (3'-phosphoadenosine 5'-phosphosulfate) cofactor.
Cytotoxic concentration	Not given
Genotoxic Effects	See remarks for results.
Appropriate statistical evaluations?	None given
Remarks for results	No mutagenic effects except a significant increase in the revertants per plate was reported for strain TA1538 in the presence of S-9 and PAPS (3'-phosphoadenosine 5'-phosphosulfate) cofactor. The authors proposed that mutagenic response was related to the formation of the sulfate ester of an active metabolite. All other strains of Salmonella typhimurium were not mutagenic in assays using PAPS.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	To L.P., Hunt T.P. and Andersen M.E. (1982) Mutagenicity of trans-anethole, estragole, eugenol and safrole in the Ames Salmonella typhimurium assay. Bulletin of Environmental Contamination and Toxicology, 28(6), 647-654.

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	Ames
Test Type	Reverse mutation
System of Testing	Bacterial

GLP	No
Year	1979
Species/Strain	*Salmonella typhimurium* TA 98, and TA 100
Metabolic Activation	Metabolic activation was provided by hepatic S13 fractions prepared from Aroclor 1254-treated CD rats
Doses/Concentration	The doses were 5-20 umoles/plate in TA100 and up to 30 umoles/plate in TA98
Statistical Methods	Not given
Remarks for Test Conditions	The vehicle and negative control was ethanol. Positive controls were not included.
Results	Equivocal. Very weak activity without metabolic activation in TA100. Activity increased in TA100 with activation. No effect was seen in TA98.
Cytotoxic concentration	Not given
Genotoxic Effects	Positive in TA100. Negative in TA98.
Appropriate statistical evaluations?	None given
Remarks for results	Very weak activity without metabolic activation in TA100. Activity increased in TA100 with activation. No effect was seen in TA98
Conclusion Remarks	Equivocal.
Data Qualities Reliabilities	Reliability code 3. Not reliable.
Remarks for Data Reliability	Code 3. Does not meet important criteria of current standard methods.
References	Swanson A.B., Chambliss D.D., Blomquist J.C., Miller E.C. and Miller J.A. (1979) The mutagenicities of safrole, estragole, eugenol, *trans*-anethole, and some of their known or possible metabolites for *Salmonella typhimurium* mutants. Mutation Research, 60(2), 142-153.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Purity 99.9%
Method/guideline	Rec assay performed according to Kada *et al*., 1980
Test Type	DNA repair
System of Testing	Bacterial
GLP	Ambiguous
Year	1982

Species/Strain	*Bacillus subtilis* H17 Rec + and M45 Rec -
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced Sprague Dawley rats
Doses/Concentration	4 mg/disk
Statistical Methods	Student's t test
Remarks for Test Conditions	Zones of killing with both strains (Rec + and Rec -) were measured and the difference between them was taken as the rec effect. Conducted according to Kada *et al*. except that 2 E5 spores used instead of 2 E6 to increase the sensitivity of the test.
Results	Negative
Cytotoxic concentration	Not given
Genotoxic Effects	None
Appropriate statistical evaluations?	None given
Remarks for results	Negative
Conclusion Remarks	The test substance did not induce DNA repair.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Sekizawa J. and Shibamoto T. (1982) Genotoxicity of safrole-related chemicals in microbial test systems. Mutation Research. 101(1), 127-140.

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	UDS
Test Type	DNA repair
System of Testing	Mammalian
GLP	Ambiguous
Year	1990
Species/Strain	Hepatocytes from Male Fisher 344 rats
Metabolic Activation	No
Doses/Concentration	0.148-1480 mg (10-6 to 10-2 M)
Statistical Methods	Not given

Remarks for Test Conditions	Unscheduled DNA synthesis was measured by determining the amount of [3H]thymidine incorporated into hepatocyte nuclear DNA during treatment of the cells with test substance.
Results	Positive. Dose related increase in UDS. 2.7 times greater than control.
Cytotoxic concentration	5 X 10-3 M
Genotoxic Effects	Positive
Remarks for results	No UDS observed at concentrations at or above 5 X 10-3 M at which there was significant LDH leakage indicating cytotoxicity.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Howes A.J., Chan V.S.W. and Caldwell J. (1990) Structure-specificity of the genotoxicity of some naturally occurring alkenylbenzenes determined by the unscheduled DNA synthesis assay in rat hepatocytes. Food and Chemical Toxicology, 28(8), 537-542.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Purity greater than 99%
Method/guideline	UDS
Test Type	DNA repair
System of Testing	Mammalian
GLP	Ambiguous
Year	1992
Species/Strain	Hepatocytes from Male Fisher 344 rats
Metabolic Activation	No
Doses/Concentration	10-4 to 10-3 M (14.8-148 mg)
Statistical Methods	Not given
Remarks for Test Conditions	Unscheduled DNA synthesis was measured by determining the amount of [3H]thymidine incorporated into hepatocyte nuclear DNA during treatment of the cells with test substance. A ratio of 1.5 is considered to be a positive response.
Results	Positive. Dose related increase in UDS. 2.68 +/- 0.93 times greater than control at 5 X 10-3 M
Cytotoxic concentration	5 X 10-3 M
Genotoxic Effects	Positive

Appropriate statistical evaluations?	Not given
Remarks for results	No UDS observed at concentrations above 5 X 10-3 M at which there was significant LDH leakage indicating cytotoxicity.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Chan V.S.W. and J. Caldwell. (1992) Comparative induction of unscheduled DNA synthesis in cultured rat hepatocytes by allylbenzenes and their 1'-hydroxy metabolites. Food and Chemical Toxicology, 30, 831-836.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Purity greater than 99%
Method/guideline	UDS
Test Type	DNA repair
System of Testing	Mammalian
GLP	Ambiguous
Year	1992
Species/Strain	Hepatocytes from Wistar rats
Metabolic Activation	No
Doses/Concentration	0.01-10 mM (1.48-1482 mg)
Statistical Methods	Not given
Remarks for Test Conditions	Unscheduled DNA synthesis was measured by determining the amount of [3H]thymidine incorporated into hepatocyte nuclear DNA during treatment of the cells with test substance. Fifty hepatocytes per slide from 3 different parallel cultures were evaluated for UDS. Results reconfirmed with independent repeat experiment. Net grain values determined by subtracting the mean of three cytoplasm grain counts from the nuclear grain counts. Cytotoxic effects qualified by determination of necrotic cells. UDS positive cells determined to be percentage of cells with five or more net grains increase over negative controls.
Results	Positive at all concentrations.
Cytotoxic concentration	1 X 10-2 M
Genotoxic Effects	Positive
Appropriate statistical evaluations?	None given

evaluations?	
Remarks for results	Positive.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Muller L. Kasper P., Muller-Tegethoff K. and Petr T. (1994) The genotoxic potential in vitro and in vivo of the allyl benzene etheric oils estragole, basil oil and trans-anethole. Mutation Research, 325(4), 129-136.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Purity greater than 99%
Method/guideline	Chromosomal aberrations in V79 cells
Test Type	Chromosomal Aberration
System of Testing	Mammalian
GLP	Ambiguous
Year	1992
Species/Strain	V79 cells from Wistar rats
Metabolic Activation	With and without rat liver microsome fraction S9 from Aroclor induced rats
Doses/Concentration	10-5 to 10-3 M (1.48 mg- 148 mg)
Statistical Methods	Not given
Remarks for Test Conditions	Chromosomal aberrations determined in V79 cells with and without metabolic activation. Cultures harvested 18 hours after treatment. (2 hour treatment with S9 mix)
Results	Negative
Genotoxic Effects	Negative
Appropriate statistical evaluations?	Chi square distribution
Remarks for results	Negative
Conclusion Remarks	Estragole did not induce chromosomal aberrations in V79 cells with and without metabolic activation.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Muller L. Kasper P., Muller-Tegethoff K. and Petr T. (1994) The genotoxic potential *in vitro* and *in vivo* of the allyl benzene etheric oils estragole, basil oil and trans-anethole. Mutation

etheric oils estragole, basil oil and trans-anethole. Mutation Research, 325(4), 129-136.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Purity 99%
Method/guideline	Rec assay performed according to Mazza *et al*., 1982
Test Type	DNA repair
System of Testing	Bacterial
GLP	Ambiguous
Year	1991
Species/Strain	*Bacillus subtilis* PB1652 and PB1791
Metabolic Activation	None
Doses/Concentration	10-30 microliters (9.6-29 micrograms/plate)
Statistical Methods	Not given
Remarks for Test Conditions	A positive DNA damaging activity was assumed when the ratio of the inhibition zone of the rec- mutant and that of the parental rec + strain exceeded the value of 1.2.
Results	Positive
Cytotoxic concentration	Not given
Genotoxic Effects	Positive
Appropriate statistical evaluations?	None given
Remarks for Results	Positive
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Zani F., Massimo G., Benvenuti S., Bianchi A., Albasini A., Melegari M., Vampa G., Bellotti A., Mazza P. (1991) Studies on the genotoxic properties of essential oils with Bacillus subtilis rec-assay and Salmonella microsome reversion assay. Planta Medica 57(3), 237-241.

4.2.2 *In vivo* Genotoxicity

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	32P-post-labelling analysis of DNA adducts
Test Type	Adduct formation
GLP	No
Year	1984
Species/Strain	Mouse/CD-1
Sex	Female
Route of Administration	Intraperitoneal
Doses/Concentration	2 or 10 mg/mouse
Exposure Period	Single dose
Remarks for Test Conditions	Groups of 3-4 female CD-1 mice were given an intraperitoneal injection of 0, 2 or 10 mg estragole/mouse in 0.1 ml trioctanoin. Twenty-four hours following treatment, mice were killed and livers were collected and frozen at -80 deg C. DNA was isolated from the frozen livers using a rapid solvent-extraction procedure and quantitated spectrophotometrically. DNA was digested and 32P-labelled. Labelled adducts were purified by reversed phase thin layer chromatography and contact transfer to polyethyleneimine-cellulose. Adduct levels (as reactive adduct labelling [RAL]) were determined (adduct spot/normal nucleotidesx600) and covalent binding indices (CBI) were calculated (umol of anethole bound/mol of DNA nucleotides divided by mmol of anethole administered/kg bw).
Genotoxic effects	Positive
NOEL (C)/ LOEL (C)	LOEL: 2 mg/kg bw
Remarks for Results	DNA adducts were detected at both dose levels.
Conclusion Remarks	Estragole showed binding potential to mouse-liver DNA.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Comparable to guideline study with acceptable restrictions.
References	Randerath, K., Haglund, R.E., Phillips, D.H., and Reddy, M.V. (1984) 32P-Post-labelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally occurring alkenylbenzenes. I. Adult female CD-1 mice. Carcinogenesis 5(12): 1613-1622.

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	32P-post-labelling analysis of DNA adducts
Test Type	Adduct formation
GLP	No
Year	1981
Species/Strain	Mouse/B6C3F1
Sex	Male and Female
Route of Administration	Intraperitoneal
Doses/Concentration	14 mg/kg bw
Exposure Period	Single dose
Remarks for Test Conditions	In a study designed to detect DNA adduct formation of estragole, 9-day old male or female B6C3F1 mice (mean weight, 6g) were given intraperitoneal injections of 0.5 mmol (14 mg/kg) of labeled estragole and sacrificed after 23 hours.
NOEL (C)/ LOEL (C)	LOEL: 14 mg/kg bw
Genotoxic effects	Positive
Remarks for Results	DNA adducts were detected.
Conclusion Remarks	Estragole showed binding potential to mouse-liver DNA.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Comparable to guideline study with acceptable restrictions.
References	Phillips, D.H., J.A. Miller, E.C. Miller, and B. Adams. (1981) Structures of the DNA adducts formed in mouse liver after administration of the proximate hepatocarcinogen 1'-hydroxyestragole. Cancer Research, 41, 176-186.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Purity 98%
Method/guideline	*in vivo* UDS
Test Type	DNA repair
GLP	Ambiguous

Year	1994
Species/Strain	Rat/Wistar
Sex	Male
Route of Administration	Gavage
Doses/Concentration	500, 1,000 or 2,000 mg/kg bw
Exposure Period	Single dose
Remarks for Test Conditions	Test material in peanut oil was administered to male Wistar rats at dose levels of 500, 1,000 or 2,000 mg/kg bw. Hepatocytes isolated from sacrificed rats 4 or 12 hours after the single dose. After 18 hours of culture, fifty hepatocytes per slide were evaluated for UDS. Net grain values obtained by subtracting the mean of three cytoplasm grain counts from the nuclear grain counts. Cytotoxic effects determined by the number of necrotic cells. Cells considered positive for UDS if percentage of cells with five or more net grains increased over the negative concurrent control values.
Genotoxic effects	500 mg/kg bw- weak effect; 1,000 mg/kg weak effect; 2,000 mg/kg clear positive effect at this dose level. No difference between cells isolated at 4 hours and those isolated at 12 hours.
NOEL (C)/ LOEL (C)	LOEL: 500 mg/kg bw
Appropriate statistical evaluations?	None given
Remarks for Results	Only a very slight increase in net grain values reported for the 500 and 1000 mg/kg bw dose levels. The highest dose levels produced clear increases.
Conclusion Remarks	The authors characterize the results seen at the two lowest dose levels as being very slight increases and given the lack of appropriate statistical analyses, these results are considered questionable.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Muller L. Kasper P., Muller-Tegethoff K. and Petr T. (1994) The genotoxic potential in vitro and in vivo of the allyl benzene etheric oils estragole, basil oil and trans-anethole. Mutation Research, 325(4), 129-136.

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	32P-post-labelling analysis of DNA adducts
Test Type	Adduct formation

GLP	No
Year	1984
Species/Strain	Mouse/B6C3F1
Sex	Male
Route of Administration	Intraperitoneal
Doses/Concentration	0.25, 0.5, 1.0, and 3.0 mmol
Exposure Period	23, 29 or 43 days
Remarks for Test Conditions	32P-post-labelling analysis was used to detect test material-DNA adducts in livers of treated mice. B6C3F1 male mice received 0.25, 0.5, 1.0 and 3.0 umol of test material on days 1, 8, 15 and 22, respectively, after birth. Groups of 3 mice were killed for analysis on days 23, 29 and 43 (i.e. 1, 7, and 21 days after the final injection) and the livers removed and weighed. Vehicle was trioctanoin.
Genotoxic effects	Positive
Remarks for Results	DNA adducts were detected.
Conclusion Remarks	Estragole showed binding potential to mouse-liver DNA.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Comparable to guideline study with acceptable restrictions.
References	Phillips D.H., Reddy M.V. and Randerath K. (1984) 32P-Post-labelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally occurring alkenylbenzenes. II. Newborn male B6C3F1 mice. Carcinogenesis, 5(12), 1623-1628.

4.3 Repeated Dose Toxicity

Substance Name	Estragole
CAS No.	140-67-0
Method/guideline	Carcinogenesis study
GLP	Ambiguous
Year	1983
Species/strain	Mice/CD-1

Sex	Male and Female
Route of Administration	Gavage
Doses/concentration Levels	0, 370 mg/kg bw
Exposure Period	Five weeks
Frequency of Treatment	Twice a week for 10 doses
Control Group	Yes
Post Exposure	13 months
Remarks for Test Conditions	Male (55) and female (49) CD-1 mice were administered 370 mg/kg of estragole by gavage twice a week for ten doses beginning at 4 days of age. The mice were weaned at 35 days of age following the last intubation.
NOAEL(NOEL)	Less than 370 mg/kg bw
LOAEL(LOEL)	370 mg/kg bw
Toxic Response/effects by Dose Level	See remarks for results
Appropriate statistical evaluations?	Yes
Remarks for Results	Hepatomas were observed as early as 11 months. At 14 months, 73% of the males (3.5 hepatomas/mouse) and 24% of control males (0.6 hepatomas/mouse) exhibited hepatomas. The incidence of hepatomas in females (9%, 0.1 hepatomas/mouse) was not statistically different from control females (2%, 0.02 hepatomas/mouse) [Miller *et al*., 1983]
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Miller, E.C., A.B. Swanson, D.H. Phillips, T.L. Fletcher, A. Liem, and J.A. Miller. (1983) Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. Cancer Research, 43, 1124-1134.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	The metabolites, 1-hydroxyestragole and estragole epoxide, were also evaluated.
Method/guideline	Carcinogenesis study
GLP	Ambiguous
Year	1983

Species/strain	Mice/CD-1
Sex	Male and Female
Route of Administration	Intraperitoneal
Doses/concentration Levels	9.45 mmol/mouse of estragole or estragole epoxide or 1.87 mmoles/mouse of 1'-hydroxyestragole by intraperitoneal injection distributed in a ratio of 1:2:4:8 on days 1, 8, 15, and 22, respectively, of life. These doses correspond to 0.63, 1.26, 2.52, and 5.04 mmol/mouse, respectively.
Exposure Period	22 days
Frequency of Treatment	Days 1, 8, 15, and 22 of life
Control Group	Yes
Post Exposure	13 months
Remarks for Test Conditions	Male (50) and female (50) CD-1 mice were administered a total dose of 9.45 mmol/mouse of estragole or estragole epoxide or 1.87 mmoles/mouse of 1'-hydroxyestragole by intraperitoneal injection distributed in a ratio of 1:2:4:8 on days 1, 8, 15, and 22, respectively, of life. These doses correspond to 0.63, 1.26, 2.52, and 5.04 mmol/mouse, respectively. The mice were weaned at 22 days of age.
Toxic Response/effects by Dose Level	See remarks for results
Appropriate statistical evaluations?	Yes
Remarks for Results	At 12 months, 65% of the mice receiving estragole exhibited hepatomas (1.7 hepatomas/mouse) versus 26% of controls (0.5 hepatomas/mouse) exhibited hepatomas. The incidence of hepatomas in mice given estragole epoxide (40%, 0.6 hepatomas/mouse) was not statistically different from control (26%, 0.5 hepatomas/mouse). For 1'-hydroxyestragole, 93% of the mice receiving the test substance (2.7 hepatomas/mouse) and 15% of control males (0.2 hepatomas/mouse) exhibited hepatomas [Miller *et al*., 1983]
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Miller, E.C., A.B. Swanson, D.H. Phillips, T.L. Fletcher, A. Liem, and J.A. Miller. (1983) Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. Cancer Research, 43, 1124-1134.

Substance Name	Estragole
CAS No.	140-67-0

Remarks for Substance	Data is for metabolite, 1-hydroxyestragole
Method/guideline	Carcinogenesis study
GLP	Ambiguous
Year	1987
Species/strain	Mice/Male C57BL/6J x C3H/HeJ F1
Sex	Male and Female
Route of Administration	Intraperitoneal
Doses/concentration Levels	Dose levels were 0.1 mmol on Day 1, 0.04 mmol on days 8 and 15, and 0.08 mmol on day 22 after birth. The levels are calculated to provide 11.7 on day 1, 18.8 on day 8, 9.3 on day 15 and 10.1 mg/kg bw on day 22, respectively.
Exposure Period	22 days
Frequency of Treatment	Days 1, 8, 15, and 22 of life
Control Group	Yes
Post Exposure	14 months
Remarks for Test Conditions	In a study using a hybrid strain of B6C3F1 mice, and the parent strain, C3H/He male and female mice and C57BL/6 male and female mice, the mice were given intraperitoneal injections of 1'-hydroxyestragole on days 1, 8, 15, and 22. Dose levels were 0.1 mmol on Day 1, 0.04 mmol on days 8 and 15, and 0.08 mmol on day 22 after birth. The levels are calculated to provide 11.7 on day 1, 18.8 on day 8, 9.3 on day 15 and 10.1 mg/kg bw on day 22, respectively. The experiment was terminated after 14 months.
Toxic Response/effects by Dose Level	See remarks for results
Appropriate statistical evaluations?	Yes
Remarks for Results	The first tumour-bearing mouse was observed at 10 months. At 12 months, 76% of the treated C3H/He male mice (3.0 hepatomas/mouse) and 26% of control mice (0.3 hepatomas/mouse) exhibited hepatomas. The incidence of hepatomas in C3H/He female mice (6% 0.06 hepatomas/mouse) was not statistically different from those of control females. For C57BL/6 mice, the incidence of hepatomas in treated males was 14% (0.3 hepatomas/mouse) and was 5% (0.07 hepatomas/mouse) in control males. No hepatomas were observed in treated or control B57BL/6 female mice
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Wiseman R.W., Miller E.C., Miller J.A. and Liem A. (1987) Structure-activity studies of the hepatocarcinogenicities of alkenylbenzene derivatives related to estragole and safrole on administration to preweanling male C57BL/6J x C3H/HeJ F1

	administration to preweanling male C57BL/6J x C3H/HeJ F1 mice. Cancer Research, 47(9), 2275-2283.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for metabolite, 1-hydroxyestragole
Method/guideline	Carcinogenesis study
GLP	Ambiguous
Year	1987
Species/strain	Mice/Male B6C3F1
Sex	Male
Route of Administration	Intraperitoneal
Doses/concentration Levels	0.10 mmol/g (15 mg/kg) and 0.01 mmol/g (1.5 mg/kg)
Exposure Period	Single dose
Frequency of Treatment	12 days after birth
Control Group	Yes
Post Exposure	12 months
Remarks for Test Conditions	Groups of male B6C3F1 mice were given single intraperitoneal injections of 0.10 mmol/g (15 mg/kg) of body weight of 1'-hydroxyestragole 12 days after birth. Animals were sacrificed after 12 months and incidence of hepatic tumors were measured. A second group of males was given a lower dose of 0.01 mmol/g of body weight.
Toxic Response/effects by Dose Level	See remarks for results
Appropriate statistical evaluations?	Yes
Remarks for Results	A statistically significant increase in the incidence of hepatomas/mouse were observed for both substances at 0.1mmol/g bw, but no significant increase was observed at the low dose of 0.01 mmol/g bw (1.5 mg/kg).
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Wiseman R.W., Miller E.C., Miller J.A. and Liem A. (1987) Structure-activity studies of the hepatocarcinogenicities of alkenylbenzene derivatives related to estragole and safrole on administration to preweanling male C57BL/6J x C3H/HeJ F1 mice. Cancer Research, 47(9), 2275-2283.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	The metabolite, 1-hydroxyestragole, was also evaluated.
Method/guideline	Carcinogenesis study
GLP	Ambiguous
Year	1983
Species/strain	Mice/CD-1
Sex	Female
Route of Administration	Oral-Diet
Doses/concentration Levels	0, 2300 or 4600 ppm for estragole and 2500 ppm for 1-hydroxyestragole
Exposure Period	12 months
Frequency of Treatment	Daily
Control Group	Yes
Remarks for Test Conditions	In a multipart study evaluating the carcinogenic potential of allylalkoxybenzene derivatives, groups of CD-1 female mice (mean weight 24 g) were maintained on a diet containing 2300 or 4600 ppm estragole or 2500 ppm 1'-hydroxy estragole. The authors estimated that the dietary levels corresponded to an average daily intake of 150-300 and 300-600 mg/kg bw for animals on the 2300 ppm and 4600 ppm estragole diet, respectively, and 180-360 mg/kg bw for animals on the 1'-hydroxyestragole diet. To avoid intolerance the dietary concentration was reduced by 75% for the first 10 days and 50% for the next 10 days. The target diet was then maintained for 12 months.
NOAEL(NOEL)	Less than 2300 ppm
LOAEL(LOEL)	2300 ppm
Actual dose received by dose level and sex	The authors estimated that the dietary levels corresponded to an average daily intake of 150-300 and 300-600 mg/kg bw for animals on the 2300 ppm and 4600 ppm estragole diet, respectively, and 180-360 mg/kg bw for animals on the 1'-hydroxyestragole diet.
Toxic Response/effects by Dose Level	See remarks for results
Appropriate statistical evaluations?	Yes
Remarks for Results	Survival at 20 months was slightly lower (68-70%) for estragole fed animals compared to control animals (78%). The average life span of mice given 1'-hydroxyestagole was 13.6 months compared to 18 months in controls. Body weights measured at

	compared to 18 months in controls. Body weights measured at 1, 4, and 8 months were markedly reduced at 4 and 8 months compared to controls. At 10 months, the incidence of hepatomas was 58% for animals at 2300 ppm estragole, 71% for animals at 4600 ppm estragole and 56% for animals at 2500 ppm of 1'-hydroxyestragole and 0 % in controls. Histopathological examinations revealed portal fibrosis, chronic inflammation and bile duct proliferation in addition to the tumours. Varied number of ceroid-laden histocytes and focal area of hyperplasia and megalocytosis were also reported. Four mice fed 4600 ppm estragole had hepatic angiosarcomas
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Miller, E.C., A.B. Swanson, D.H. Phillips, T.L. Fletcher, A. Liem, and J.A. Miller. (1983) Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. Cancer Research 43, 1124-1134.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for structurally related alkoxybenzene derivative, methyl eugenol. Purity greater than 99%
Method/guideline	National Toxicology Program. Toxicology and Carcinogenesis study NTP TR 491
GLP	Yes
Year	1998
Species/strain	Rat/F344/N
Sex	Male and Female
Route of Administration	Oral-Gavage
Doses/concentration Levels	0, 37, 75, or 150 mg/kg bw/d; stop exposure group 300 mg/kg bw/d
Exposure Period	105 weeks
Frequency of Treatment	Daily (5 days/week)
Control Group	Yes
Post Exposure	52 weeks for the stop exposure group
Remarks for Test Conditions	Groups of fifty male and fifty female rats each were administered 0, 37, 75 or 150 mg/kg bw/d methyl eugenol in 0.5% methyl cellulose via gavage once per day, five days a week for 105 weeks. Animals were housed five per cage and fed ad libitum. The animals were observed twice per day and weighed once per week for 12 weeks and once per month thereafter. Necropsies were performed on all animals.

	thereafter. Necropsies were performed on all animals. Histological examinations were performed on all animals dying during the study; all vehicle control; all low dose female rats and all high dose animals. Tissues examined included adrenal glands, brain, cecum, colon, costochondral junction, duodenum, epididymus/seminal vesicles/tunica vaginalis/scrotal sac/prostrate/testes or ovaries/uterus, esophagus, eyes, femur or sternebrae or vertebrae including marrow, gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, larynx and pharynx, liver, lungs and bronchi, mammary gland, mandibular or mesenteric lymph nodes, nasal cavity and turbinates, oral cavity, pancreas, parathyroids, pituitary gland, preputial or clitoral gland, rectum, salivary glands, sciatic nerve, skin, spinal cord, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, urinary bladder and Zymbal gland. Tissues examined in low dose male rat groups included adrenal glands, kidney, liver, spleen, and testis.
NOAEL(NOEL)	Undetermined
LOAEL(LOEL)	37 mg/kg bw/d
Toxic Response/effects by Dose Level	See remarks for results.
Appropriate statistical evaluations?	Yes
Remarks for results	All 150 and 300 mg/kg males died before the end of the study. Mean body weights of all dosed groups were less than those of the vehicle controls throughout the study. The incidences of liver non-neoplastic lesions in dosed groups of male and females were increased at 6 months, 12 months, and 2 years. There were statistically significant increases in oval cell hyperplasia, hepatocyte hypertrophy, and eosinophilic foci, at all dose levels in male and female rats. At the three highest doses (75, 150, and 300 mg/kg bw per day) atypical focal bile duct hyperplasia, focal cystic degeneration, and mixed cell foci were observed, more in males than females. Many of the same non-neoplastic lesions of the liver were reported in the 300 mg/kg bw groups of male and female rats at both 6 and 12 months in the stop-exposure group. Non-neoplastic lesions of the glandular stomach included statistically significant increases in mucosal atrophy at all dose levels and neuroendocrine hyperplasia at the three highest dose levels in females and at all dose levels in males. There was a significant increase in the incidence of nephropathy in females at 300 mg/kg, and the incidence of renal tubule hyperplasia was greater in the greater than 75 mg/kg groups than in the vehicle control. Methyl eugenol-related liver neoplasms occurred in all dosed groups and comprised hepatocellular adenomas and carcinomas, hepatocholangiomas, and hepatocholangiocarcinomas. There was a statistically significant increase (P equals 0.049 in males and P equals 0.017 in females at 37 mg/kg bw; P less than 0.001 for all other treated groups) in the incidence of hepatocellular adenomas and carcinomas in all dose groups of males and female rats. Hepatocholangiomas and hepatocholangiocarcinomas were

	Hepatocholangiomas and hepatocholangiocarcinomas were reported in the 150 mg/kg bw group of males (2/50, 4%) and females (3/49, 6%) and at higher incidence in the 300 mg/kg bw stop-exposure groups of males (13/50, 26%) and females (17/50, 34%). The appearance of cholangiocarcinomas and bile duct dysplasia was said to provide some additional evidence of carcinogenicity based on the rarity of these lesions in F344/N rats (historical incidence, 3/2145, 0.1%).
	Both benign (3/50, 6%) and malignant (4/50, 8%) neuroendocrine cell neoplasms of the glandular stomach were reported in males at 150 mg/kg bw and in the 300 mg/kg bw stop-exposure group (2/49, 4.1% benign and 2/49, 4.1% malignant). The incidence of these neoplasms was much higher in females at dose levels of 75 mg/kg bw (13/50, 26% benign and 12/50, 24% malignant) and greater.
	There were also significant increases in the incidence of: malignant mesothelioma in male rats given greater than 150 mg/kg; and of mammary gland fibroadenoma in 75 and 150 mg/kg males; and fibroma of the subcutaneous tissue in 37 and 75 mg/kg males. These neoplasms were not found in female rats at any dose level.
Conclusion Remarks	The authors determined that under the conditions of these 2-year gavage studies there was clear evidence of carcinogenic activity of methyl eugenol as shown by increased incidences of liver neoplasms and neuroendocrine tumors of the glandular stomach in male and female rats and the increased incidences of kidney neoplasms, malignant mesothelioma, mammary gland fibroadenoma, and subcutaneous fibroma and fibroma or fibrosarcoma in male rats. However, because of the evidence of toxicity of methyl eugenol in all groups of rats and mice, the study cannot be recognized as conclusive for carcinogenicity at lower, non-toxic doses. In particular, the hepatic damage undoubtedly altered the metabolism of the compound, and the gastric damage probably altered its absorption.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	National Toxicology Program (NTP) (2000) Toxicology and carcinogenesis studies of estragole in F344/N Rats and B6C3F1 mice. NTP-TR-491. U.S. Dept of Health and Human Services. NIH Publication No. 98-3950.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for structurally related alkoxybenzene derivative, methyl eugenol. Purity greater than 99%

Method/guideline	National Toxicology Program. Toxicology and Carcinogenesis study NTP TR 347
GLP	Yes
Year	1998
Species/strain	Mice/B6C3F1
Sex	Male and Female
Route of Administration	Oral-Gavage
Doses/concentration Levels	0, 37, 75, or 150 mg/kg bw/d
Exposure Period	104 weeks
Frequency of Treatment	Daily (5 days/week)
Control Group	Yes
Remarks for Test Conditions	Groups of fifty male and fifty female mice each were administered 0, 37, 75 or 150 mg/kg bw/d methyl eugenol in 0.5% methyl cellulose via gavage once per day, five days a week for 104 weeks. Animals were housed five per cage and fed ad libitum. The animals were observed twice per day and weighed once per week for 12 weeks and once per month thereafter. Necropsies were performed on all animals. Histological examinations were performed on all animals dying during the study, all vehicle controls, and all high dose animals. Tissues examined included adrenal glands, brain, cecum, colon, costochondral junction, duodenum, epididymus/seminal vesicles/tunica vaginalis/scrotal sac/prostrate/testes or ovaries/uterus, esophagus, eyes, femur or sternebrae or vertebrae including marrow, gallbladder, gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, larynx and pharynx, liver, lungs and bronchi, mammary gland, mandibular or mesenteric lymph nodes, nasal cavity and turbinates, oral cavity, pancreas, parathyroids, pituitary gland, preputial or clitoral gland, rectum, salivary glands, sciatic nerve, skin, spinal cord, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, urinary bladder and Zymbal gland.
NOAEL(NOEL)	NEED
LOAEL(LOEL)	37 mg/kg bw/d (females); NEED FROM FINAL REPORT
Toxic Response/effects by Dose Level	See remarks for results
Appropriate statistical evaluations?	Yes
Remarks for Results	Survival of all dosed groups of male mice was similar to that of the vehicle controls. The survival of treated females was significantly less than those reported for control animals. Mean body weights of dosed mice were reported to be "generally less than those of the vehicle controls throughout the studies". In female mice and, to a lesser extent, in male mice there was evidence of hepatotoxicity of methyl eugenol. Significant

	evidence of hepatotoxicity of methyl eugenol. Significant increases in oval cell hyperplasia, eosinophilic foci, hepatocyte hypertrophy and necrosis, haematopoietic cell proliferation, haemosiderin pigmentation, and bile duct cysts were observed at all dose levels in male and female mice. Non-neoplastic lesions of the glandular stomach included statistically significant increases in hyperplasia, ectasia, atrophy at all dose levels in both males and females and mineralization and necrosis in lower incidence also in both sexes incidences of chronic atrophic gastritis was high. Gastric tumours were found in two high dose males. The incidence of hepatocellular adenomas, hepatocellular carcinomas and hepatoblastomas was high in both treated and control male and female mice. While control males and females showed tumour rates of 63% (31/49) and 50% (25/50), respectively, and all treatment groups of males and females had tumour rates in excess of 92% with the exception of high dose male rates in which the tumour rate was 82% (41/50). Evidence of infection by *H. hepaticus* was found by PCR-RFLP, but associated hepatitis was not found.
Conclusion Remarks	The authors determined that under the conditions of these 2-year gavage studies there was no evidence of carcinogenic activity of d-limonene for male or female B6C3F1 mice at the dose levels tested.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	National Toxicology Program (NTP) (2000) Toxicology and carcinogenesis studies of estragole in F344/N Rats and B6C3F1 mice. NTP-TR-491. U.S. Dept of Health and Human Services. NIH Publication No. 98-3950.

4.4 Reproductive Toxicity

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for p-(2-propenyl)anisole (*trans*-anethole)
Method/Guideline	4-Generation reproduction study
Test Type	Reproductive toxicity
GLP	No
Year	1971
Species/Strain	Rat/Wistar SPF

Sex	Male and Female
Route of Administration	Oral-Diet
Duration of Test	Four generations with a minimum exposure to the treated diet of 70 days from time of weaning
Doses/Concentration	1% in the diet (approximately 600-1,500 mg/kb bw/day)
Premating Exposure period for males	F0: 70 days F1-F4: raised on treated diet
Premating Exposure period for females	F0: 70 days F1-F3: raised on treated diet
Frequency of Treatment	Daily
Control Group and Treatment	Basal diet
Remarks for Test Conditions	Groups of 20 male and 20 female Wistar SPF rats were fed 0 or 1% anethole in the diet (~600-1,500 mg/kg bw/day) for 70 days prior to mating. Four paired groups were formed: (1) control males X control females; (2) control males X treated females; (3) treated males X control females; and (4) treated males X treated females. During the mating period of 15 days, the first 3 groups were maintained on basal diet; whereas, group 4 received treated diet. During gestation and lactation, females of groups 2, 3 and 4 were maintained on 1% anethole diet. Offspring from groups 1 and 4 were used for propagating the next generation and were raised on the same dietary treatment as their parents (70 days from time of weaning). At approximately 3 months of age, rats were bred to obtain the next generation. A similar procedure was followed to obtain the 3rd and 4th generations. The treatment groups for F1, F2 and F3 were: (1) control males X control females; and (2) treated males X treated females. Mortality, body weight, food consumption, and reproductive performance (fertility, sex ratio, date of birth, stillbirths, clinical observations, litter size, litter viability) were monitored.
Actual dose received by dose level and sex	Approximately 600 to 1,500 mg/kg bw/day
Parental data and F1 as appropriate	F0: death of 1 control male and 1 treated female, no other deaths, decreased body weight in treated rats, decreased food consumption in treated rats, no effect on reproductive performance F1: no deaths, reduced body weight gain and body weight in treated rats, reduced food consumption in treated rats for 1st 2 weeks, no effect on reproductive performance
Offspring toxicity F1 and F2	F2 and F3: no deaths, reduced body weight gain and body weight in treated rats, reduced food consumption in treated rats for 1st 2 weeks, no effect on reproductive performance
Appropriate statistical evaluations?	Yes, one factor variance analysis, Fischer test, t-test, Chi-square test

Remarks for Results	The reduced palatability of the diet was considered to be responsible for the lower body weight gain and body weights of the rats receiving anethole.
Conclusion remarks	*trans*-Anethole did not affect the reproductive performance of rats over 4 generations.
Data Reliabilities Qualities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Le Bourhis B. (1973) 4-Generation reproduction study in rats given trans-anethole in the diet. Unpublished report by Sophie Holm. Laboratoire de Physiologie, Institut de Recherches appliquees aux Boissons, Montreuil, 93, France.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for p-(2-propenyl)anisole (*trans*-anethole)
Method/Guideline	Cross-fostering
Test Type	Reproductive toxicity
GLP	No
Year	1971
Species/Strain	Rat/Wistar SPF
Sex	Male and Female
Route of Administration	Oral-Diet
Duration of Test	One generation
Doses/Concentration	1% in the diet (approximately 600-1,500 mg/kb bw/day)
Premating Exposure period for males	Control F1 males from 4-generation portion of study
Premating Exposure period for females	Control and treated F1 females from 4-generation portion of study
Frequency of Treatment	Daily
Control Group and Treatment	Basal diet
Remarks for Test Conditions	In a cross-fostering experiment, groups of 6 control and 6 treated F1 females (receiving 1% anethole in the diet) were mated with control F1 males (from 4-generation portion of study). Litters born from treated females were exchanged with litters from control females at birth and reared by the new dams. Body weight and growth of pups was monitored.
Actual dose received by dose level and sex	Approximately 600-1,500 mg/kb bw/day

Parental data and F1 as appropriate	F1: no significant difference in body weights of pups from those nursed by mothers of the same group, regardless from which group they were born; final body weights of pups born from treated dams but raised by control dams regained normal values by day 28
Appropriate statistical evaluations?	Yes, one factor variance analysis, Fischer test, t-test, Chi-square test
Remarks for Results	Reduced palatability of diets containing anethole was considered an issue in the nutritional status of the dams.
Conclusion remarks	The results indicate that postnatal growth is not directly affected by anethole exposure, but is a result of the nutritional status of the dams.
Data Reliabilities Qualities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Le Bourhis, B. (1973) 4-Generation reproduction study in rats given trans-anethole in the diet. Unpublished report by Sophie Holm. Laboratoire de Physiologie, Institut de Recherches appliquees aux Boissons, Montreuil, 93, France.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for oil of nutmeg containing 10-20% p-allylalkoxybenzene derivatives, myristicin, elemicin, safrole, and methyl eugenol.
Test Type	One generation
GLP	No
Year	1973
Species/Strain	Mouse/CD-1 outbred
Sex	Female
Route of Administration	Oral-Gavage
Duration of Test	Days 6 to 15 of gestation
Doses/Concentration	0(control), 6, 26, 120, 560 mg/kg bw/day and a positive control of 150 mg/kg bw/day of aspirin.
Premating Exposure period for males	None
Premating Exposure period for females	None
Frequency of Treatment	Daily
Control Group and Treatment	Control group received corn oil vehicle (10 ml//kg); Positive control received 150 mg/kg bw/day of aspirin in corn oil.

Remarks for Test Conditions	Study measured parameters for reproductive and developmental toxicity. In the reproductive segment of the study, virgin adult female CD-1 outbred mice were gang-housed in plastic disposable cages in a temperature- and humidity-controlled room. Animals were given free access to food and fresh tap water. There were mated with untreated young adult males and observation of vaginal sperm plugs was considered day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, females were given 0, 6, 26, 120, or 560 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil. A positive control group received 150 mg/kg bw/day of aspirin. Body weights were recorded on days 0, 6, 11, 15, and 17 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On Day 17 all dams were subjected to Caesarian section and the number of implantation sites, resorption sites, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, gross pathology incidence of the dam urogenital tract, number of implantation sites, number of corpora lutea, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported. The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10x magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects.
NOAEL(NOEL)	560 mg/kg bw/day
Actual dose received by dose level and sex	560 mg/kg bw/day
Parental data and F1 as appropriate	Data for number of females mated/pregnant at each dose level: 0 mg/kg bw, 24/21; 150 mg/kg bw of aspirin, 30/20; 6 mg/kg bw, 30/22; 26 mg/kg bw, 31/21;120 mg/kg bw, 22/21; 560 mg/kg bw, 32/20. All pregnant females survived to sacrifice on Day 17. There was no significant difference in dam body weights between controls and any test group measured at Days 0, 6, 11, 15, or 17 of the study. None of the pregnant females died or aborted before Day 17and all litters were alive on Day 17 sacrifice. Average number of corpora lutea/dam mated were similar for controls and treatment groups: 0 mg/kg bw, 12.5; 150 mg/kg bw aspirin, 12.0; 6 mg/kg bw, 12.3; 26 mg/kg bw, 11.2; 120 mg/kg bw, 12.9; 560 mg/kg bw, 11.2. The average number of implantation sites/dam and % partial resorptions were similar for all groups:0 mg/kg bw, 11.8 and 19%; 150 mg/kg bw aspirin, 11.3 and 45%; 6 mg/kg bw, 12.5 and 45%; 26 mg/kg bw, 11.9 and 28%; 120 mg/kg bw, 10.5 and 28%; 560 mg/kg bw, 11.0 and 25%. Based on bodyweight changes, clinical observation, and gross examination of the urogenital tract, was no evidence of toxicity to dams.
Offspring toxicity F1 and F2	Based on gross examination of live pups, visceral examination and skeletal examination there were no signs of toxicity to offspring. The total number of live fetuses, average number of live fetuses per dam, sex ratio, number of dead fetuses, and average fetal weight were not different between control and

	average fetal weight were not different between control and treatment groups. Total number of live fetuses/dead
Conclusion remarks	The administration of up to and including 560 mg/kg bw/day of test article FDA 71-28 to pregnant mice on days 6 through 15 of gestation had no effects on nidation, maternal survival or fetal survival. The number and types of abnormalities seen in tissues of the dam or pups of the test groups did not differ for the number and type occurring spontaneously in the positive or negative controls.
Data Reliabilities Qualities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Morgareidge K. (1973a) Teratologic evaluation of FDA 71-28 in mice. Contract No. FDA 71-260. Unpublished report.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for oil of nutmeg containing 10-20% p-allylalkoxybenzene derivatives, myristicin, elemicin, safrole, and methyl eugenol
Test Type	One generation
GLP	No
Year	1973
Species/Strain	Hamster/adult golden
Sex	Female
Route of Administration	Oral-Gavage
Duration of Test	Days 6 to 10 of gestation
Doses/Concentration	0(control), 6, 28, 130, or 600 mg/kg bw/day and a positive control of 250 mg/kg bw/day of aspirin
Premating Exposure period for males	None
Premating Exposure period for females	None
Frequency of Treatment	Daily
Control Group and Treatment	Control group received corn oil vehicle (10 ml//kg); Positive control received 250 mg/kg bw/day of aspirin in corn oil.
Remarks for Test Conditions	Study measured parameters for reproductive and developmental toxicity. In the reproductive segment of the study, groups (26-28/dose/group) of virgin adult female hamster were individually housed in mess-bottom cages in a temperature- and humidity-controlled room. Animals were given free access to food and fresh tap water. There were mated one to one with untreated adult males and the appearance of motile

	to one with untreated adult males and the appearance of motile sperm in the vaginal sperm was considered day 0 of gestation. Beginning on Day 6 and continuing daily through Day 10 of gestation, females were given 0, 6, 28, 130, or 600 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil. A positive control group received 250 mg/kg bw/day of aspirin. Body weights were recorded on days 0, 8, 10, and 14 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On Day 14 all dams were subjected to Caesarian section and the number of implantation sites, resorption sites, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, gross pathology incidence of the dam urogenital tract, number of implantation sites, number of corpora lutea, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported. The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10x magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects.
NOAEL(NOEL)	600 mg/kg bw/day
Actual dose received by dose level and sex	600 mg/kg bw/day
Parental data and F1 as appropriate	Data for number of females mated/ pregnant at each dose level: 0 mg/kg bw, 27/21; 250 mg/kg bw of aspirin, 26/19; 6 mg/kg bw, 28/19; 28 mg/kg bw, 26/21; 130 mg/kg bw, 28/20; 600 mg/kg bw, 27/23. All pregnant females survived to sacrifice on Day 14. There was no significant difference in dam body weights between controls and any test group measured at Days 0, 6, 8, 10, or 14 of the study. One death each was reported in the two control groups and in the two highest dose groups before day 14. All litters were alive on Day 14 sacrifice. Average number of corpora lutea/dam mated were similar for controls and treatment groups: 0 mg/kg bw, 10.3; 250 mg/kg bw aspirin, 9.9; 6 mg/kg bw, 9.6; 28 mg/kg bw, 11.4; 130 mg/kg bw, 9.6; 600 mg/kg bw, 11.2. The average number of implantation sites/dam and % partial resorptions were similar for all groups:0 mg/kg bw, 11.7 and 15%; 250 mg/kg bw aspirin, 11.3 and 39%; 6 mg/kg bw, 12.1 and 32%; 28 mg/kg bw, 11.9 and 38%; 130 mg/kg bw, 11.5 and 42%; 600 mg/kg bw, 12.1 and 23%. Based on bodyweight changes, clinical observation, and gross examination of the urogenital tract, was no evidence of toxicity to dams.
Offspring toxicity F1 and F2	Based on gross examination of live pups, visceral examination, and skeletal examination there were no signs of toxicity to offspring in either the control or test groups. The total number of live fetuses, average number of live fetuses per dam, sex ratio, and average fetal weight were not different between control and treatment groups. A small number of dead fetuses
Conclusion remarks	The administration of up to and including 600 mg/kg bw/day of test article FDA 71-28 to pregnant golden hamsters on days 6 through 10 of gestation had no effects on nidation, maternal survival or fetal survival. The number and types of

	survival or fetal survival. The number and types of abnormalities seen in tissues of the dam or pups of the test groups did not differ for the number and type occurring spontaneously in the positive or negative controls.
Data Reliabilities Qualities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Morgareidge K. (1973b) Teratologic evaluation of FDA 71-28 in hamsters. Contract No. FDA 71-260. Unpublished report.

Substance Name	Estragole
CAS No.	140-67-0
Test Type	One generation
GLP	No
Year	1973
Species/Strain	Rat/adult Wistar
Sex	Female
Route of Administration	Oral-Gavage
Duration of Test	Days 6 to 14 of gestation
Doses/Concentration	0(control), 3, 12, 56, or 260 mg/kg bw/day and a positive control of 250 mg/kg bw/day of aspirin.
Premating Exposure period for males	None
Premating Exposure period for females	None
Frequency of Treatment	Daily
Control Group and Treatment	Control group received corn oil vehicle (10 ml//kg); Positive control received 250 mg/kg bw/day of aspirin in corn oil.
Remarks for Test Conditions	Study measured parameters for reproductive and developmental toxicity. In the reproductive segment of the study, virgin adult female Wistar were individually housed in mess-bottom cages in a temperature- and humidity-controlled room. Animals were given free access to food and fresh tap water. There were mated with untreated young adult males and observation of vaginal sperm plugs was considered day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, females were given 0, 3, 2, 56, or 260 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil. A positive control group received 250 mg/kg bw/day of aspirin. Body weights were recorded on days 0, 6, 11, 15, and 20 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On Day 20 all dams were

	anorexia in pregnant females. On Day 20 all dams were subjected to Caesarian section and the number of implantation sites, resorption sites, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, gross pathology incidence of the dam urogenital tract, number of implantation sites, number of corpora lutea, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported. The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10x magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects.
NOAEL(NOEL)	260 mg/kg bw/day
Actual dose received by dose level and sex	260 mg/kg bw/day
Parental data and F1 as appropriate	Data for number of females mated/ pregnant at each dose level: 0 mg/kg bw, 25/23; 250 mg/kg bw of aspirin, 25/22; 3 mg/kg bw, 25/25; 12 mg/kg bw, 25/23; 56 mg/kg bw, 25/22; 260 mg/kg bw, 25/21. All pregnant females survived to sacrifice on Day 20. There was no significant difference in dam body weights between controls and any test group measured at Days 0, 6, 11, 15, or 20 of the study. None of the pregnant females died or aborted before Day 20 and all litters were alive on Day 20 sacrifice. Average number of corpora lutea/dam mated were similar for controls and treatment groups: 0 mg/kg bw, 12.8; 250 mg/kg bw aspirin, 11.1; 3 mg/kg bw, 12.7; 12 mg/kg bw, 12.5; 56 mg/kg bw, 11.6; 260 mg/kg bw, 10.7. The average number of implantation sites/dam and % partial resorptions were similar for all groups:0 mg/kg bw, 11.9 and 9%; 250 mg/kg bw aspirin, 11.1 and 32%; 3 mg/kg bw, 12 and 12%; 12 mg/kg bw, 11.8 and 4%; 56 mg/kg bw, 11.1 and 5%; 260 mg/kg bw, 11.1 and 5%. Based on bodyweight changes, clinical observation, and gross examination of the urogenital tract, there was no evidence of toxicity to dams.
Offspring toxicity F1 and F2	Based on gross examination of live pups, visceral examination, and skeletal examination there were no signs of toxicity to offspring in either the control or test groups. The total number of live fetuses, average number of live fetuses per dam, sex ratio, and average fetal weight were not different between control and treatment groups. A small number of dead fetuses
Conclusion Remarks	The administration of up to and including 260 mg/kg bw/day of test article FDA 71-28 to pregnant Wistar rats on days 6 through 15 of gestation had no effects on nidation, maternal survival or fetal survival. The number and types of abnormalities seen in tissues of the dam or pups of the test groups did not differ for the number and type occurring spontaneously in the positive or negative controls.
Data Reliabilities Qualities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Morgareidge K. (1973c) Teratologic evaluation of FDA 71-28 in rats. Contract No. FDA 71-260. Unpublished report.

4.5 Developmental/Teratogenicity Toxicity

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for p-(2-propenyl)anisole (*trans*-anethole)
Test Type	Developmental toxicity
GLP	Yes
Year	1992
Species/strain	Rat/Crl:CDBR VAF/Plus (Sprague-Dawley)
Sex	Female
Route of Administration	Oral-Gavage
Duration of Test	Approximately 32 days
Doses/concentration Levels	0, 35, 175, or 350 mg/kg bw/day
Exposure Period	Approximately 32 days
Frequency of Treatment	Daily
Control Group and Treatment	Corn oil vehicle
Remarks for Test Conditions	Groups of 10 female rats were gavaged with anethole at 0, 35, 175, or 350 mg/kg bw/day in corn oil for 7 days prior to co-habitation with male rats until day 4 of lactation for those rats producing litters and day 25 of cohabitation for those rats without confirmed mating dates. Body weight and feed consumption was monitored. Fertility, gestation index, implantation sites, length of gestation, number of stillborn pups, litter size, pup viability, pup weight, and clinical observations of pups were recorded. On day 4 of lactation, pups were examined, killed, and discarded.
NOAEL(NOEL) maternal toxicity	35 mg/kg bw/day
LOAEL(LOEL) maternal toxicity	175 mg/kg bw/day
NOAEL (NOEL) developmental toxicity	175 mg/kg bw/day
LOAEL (LOEL) developmental toxicity	350 mg/kg bw/day

Actual dose received by dose level and sex	0, 35, 175, or 350 mg/kg bw/day
Maternal data with dose level	At 350 mg/kg bw/day: significantly reduced mean body weight and feed consumption throughout study; 1 rat found dead on day 20 of gestation (necropsy showed congested lungs, but uterine contents showed 17 normal fetuses and 2 early resorptions); 2 rats had urine-stained abdominal fur during the premating period, one of these rats also "had a tan perivaginal substance and appeared pale on day 23 of gestation, and during lactation was emaciated and pale and had an ungroomed coat and red perioral and perivaginal substances"; in necropsy 1 rat had a raised yellow area in the liver, 1 rat had hematomas on the vessels supplying the implantation sites; average gestation duration was increased (number of dams delivering on days 23 and 24 was increased over controls); number of dams with stillborn pups and with all pups dying before postpartum day 4 was significantly increased (P less than or equal to 0.01). At 175 mg/kg bw/day, mean body weight was significantly decreased on gestation days 6 and 14; feed consumption was significantly reduced during premating days 1-8 but not during gestation
Fetal Data with Dose Level	At 350 mg/kg bw/day, number of liveborn pups (75) was significantly decreased (P less than or equal to 0.01) compared to controls (147), number of stillborn pups (18) was significantly increased (P less than or equal to 0.01) compared to controls (0),number of pups dying on day 1 and days 2-4 (8 and 7 respectively) was significantly increased (P less than or equal to 0.01) compared to controls (0 and 0, respectively), viability index (number of live pups on postpartum day 4/number of liveborn pups on postpartum day 1) was significantly (P less than or equal to 0.01) decreased (80%) compared to controls (99.3%); number of surviving pups/litter on postpartum day 4 (7.5) was significantly (P less than or equal to 0.01) decreased compared to controls (14.6); live litter size on postpartum day 4 (12.0) was significantly (P less than or equal to 0.05) decreased compared to controls (14.6); pup weight/litter on postpartum day 1 (5.1 g) was significantly (P less than or equal to 0.05) decreased compared to controls (6.2 g). No other effects were reported at the other doses. No anomalies were reported.
Appropriate statistical evaluations	Yes, Bartlett's Test, ANOVA, Dunnett's test, Kruskal-Wallis Test, Dunn's test, Fischer's Test
Conclusion Results	Anethole did not cause any developmental effects on the rat fetus at doses below those causing maternal toxicity (reduced body weight and feed consumption).
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Argus Research Laboratories, Inc (1992) Reproductive and developmental toxicity screening test of (anethole) administered orally *via* gavage to Crl:CDBR VAF/Plus female rats. Final Report.

Report.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for oil of nutmeg containing 10-20% p-allylalkoxybenzene derivatives, myristicin, elemicin, safrole, and methyl eugenol
Test Type	Teratology study
GLP	No
Year	1973
Species/strain	Mouse/CD-1 outbred
Sex	Female
Route of Administration	Oral-Gavage
Duration of Test	10 days
Doses/concentration Levels	0(control), 6, 26, 120, 560 mg/kg bw/day and a positive control of 150 mg/kg bw/day of aspirin
Exposure Period	Days 6 to 15 of gestation
Frequency of Treatment	Daily
Control Group and Treatment	Control group received corn oil vehicle (10 ml//kg); Positive control received 150 mg/kg bw/day of aspirin in corn oil
Remarks for Test Conditions	Study measured parameters for reproductive and developmental toxicity. In the study, virgin adult female CD-1 outbred mice were gang-housed in plastic disposable cages in a temperature- and humidity-controlled room. Animals were given free access to food and fresh tap water. There were mated with untreated young adult males and observation of vaginal sperm plugs was considered day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, groups (20-22/group) of pregnant females were given 0, 6, 26, 120, or 560 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil. A positive control group received 150 mg/kg bw/day of aspirin. Body weights were recorded on days 0, 6, 11, 15, and 17 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On Day 17 all dams were subjected to Caesarian section and the number of implantation sites, resorption sites, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, gross pathology incidence of the dam urogenital tract, number of implantation sites, number of corpora lutea, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported (these data were described in the robust summary for reproductive effects for the test material). The urogenital tract of each dam was examined for anatomical

	urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10x magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects (the maternal and developmental fetal effects are discussed in this robust summary).
NOAEL(NOEL) maternal toxicity	560 mg/kg bw/day
NOAEL (NOEL) developmental toxicity	560 mg/kg bw/day
Actual dose received by dose level and sex	0, 6, 26, 120, or 560 mg/kg bw of the test material (FDA 71-28)
Maternal data with dose level	Daily clinical observation and measurement of body weight gain failed to show any differences between control and test groups of female mice. The number pregnant and % pregnancy were similar for all dose and control groups. No abortions were observed in any group.
Fetal Data with Dose Level	The average fetal weight of treatment and control groups were not statistically different ($p>0.05$). The total number of live fetuses were similar for test and control groups. Also, there was no significant difference in the number of dead fetuses between test and control groups. Skeletal examination of sternebrae showed no significant differences in the incidence of incomplete ossification or missing sternebrae for test and control groups. Likewise the incidences of fetuses with more than 13 ribs, incomplete ossification of vertebrae and extremities, incomplete skull closure was similar for test and control animals. Visceral examination failed to reveal any evidence of abnormalities at any dose level.
Conclusion Results	There was no evidence of maternal toxicity or developmental toxicity at dose levels up to and including 560 mg/kg bw/day of test material.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Morgareidge K. (1973a) Teratologic evaluation of FDA 71-28 in mice. Contract No. FDA 71-260. Unpublished report.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for oil of nutmeg containing 10-20% p-allylalkoxybenzene derivatives, myristicin, elemicin, safrole, and methyl eugenol
Test Type	Teratology study
GLP	No

Year	1973
Species/strain	Rat/female Wistar
Sex	Female
Route of Administration	Oral-Gavage
Duration of Test	10 days
Doses/concentration Levels	0(control), 3, 12, 56, 260 mg/kg bw/day and a positive control of 250 mg/kg bw/day of aspirin
Exposure Period	Days 6 to 15 of gestation
Frequency of Treatment	Daily
Control Group and Treatment	Control group received corn oil vehicle (10 ml//kg); Positive control received 250 mg/kg bw/day of aspirin in corn oil
Remarks for Test Conditions	Study measured parameters for reproductive and developmental toxicity. In the study, virgin adult female rats were individually housed in mess bottom cages in a temperature- and humidity-controlled room. Animals were given free access to food and fresh tap water. There were mated with untreated young adult males and observation of vaginal sperm plugs was considered day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, groups (21-25/group) of pregnant females were given 0, 6, 26, 120, or 260 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil. A positive control group received 250 mg/kg bw/day of aspirin. Body weights were recorded on days 0, 6, 11, 15, and 20 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On Day 20 all dams were subjected to Caesarian section and the number of implantation sites, resorption sites, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, gross pathology incidence of the dam urogenital tract, number of implantation sites, number of corpora lutea, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported (these data were described in the robust summary for reproductive effects for the test material). The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10x magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects (the maternal and developmental fetal effects are discussed in this robust summary).
NOAEL(NOEL) maternal toxicity	260 mg/kg bw/day
NOAEL (NOEL) developmental toxicity	260 mg/kg bw/day
Actual dose received by dose level and sex	0, 3, 12, 56, or 260 mg/kg bw of the test material (FDA 71-28)

Maternal data with dose level	Daily clinical observation and measurement of body weight gain failed to show any differences between control and test groups of female rats. The number pregnant and % pregnancy were similar for all dose and control groups. No abortions were observed in any group.
Fetal Data with Dose Level	The average fetal weight of treatment and control groups were not statistically different ($p>0.05$). The total number of live fetuses were similar for test and control groups. Also, there was no significant difference in the number of dead fetuses between test and control groups. Except for positive control group, skeletal examination of sternebrae showed no significant differences in the incidence of incomplete ossification or missing sternebrae for test and untreated control group. Likewise the incidences of fetuses with more than 13 ribs, incomplete ossification of vertebrae and extremities, incomplete skull closure were similar for test and the untreated control group except for the positive aspirin-treated control group in which increases in incidences of these skeletal effects were observed. Visceral examination failed to reveal any evidence of abnormalities at any dose level.
Conclusion Results	There was no evidence of maternal toxicity or developmental toxicity at dose levels up to and including 260 mg/kg bw/day of test material.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Morgareidge K. (1973c) Teratologic evaluation of FDA 71-28 in rats. Contract No. FDA 71-260. Unpublished report.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for oil of nutmeg containing 10-20% p-allylalkoxybenzene derivatives, myristicin, elemicin, safrole, and methyl eugenol
Test Type	Teratology study
GLP	No
Year	1973
Species/strain	Hamster/female golden
Sex	Female
Route of Administration	Oral-Gavage
Duration of Test	5 days
Doses/concentration Levels	0(control), 6, 28, 130, 600 mg/kg bw/day and a positive control of 250 mg/kg bw/day of aspirin

Exposure Period	Days 6 to 10 of gestation
Frequency of Treatment	Daily
Control Group and Treatment	Control group received corn oil vehicle (10 ml//kg); Positive control received 250 mg/kg bw/day of aspirin in corn oil
Remarks for Test Conditions	Study measured parameters for reproductive and developmental toxicity. In the study, virgin adult female hamsters were individually housed in mess bottom cages in a temperature- and humidity-controlled room. Animals were given free access to food and fresh tap water. There were mated one to one with untreated young adult males and the appearance of motile sperm in the vaginal sperm was considered day 0 of gestation. Beginning on Day 6 and continuing daily through Day 10 of gestation, groups (19-23/group) of pregnant females were given 0, 6, 28, 130, or 600 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil. A positive control group received 250 mg/kg bw/day of aspirin. Body weights were recorded on days 0, 6, 8, 10, and 14 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On Day 14 all dams were subjected to Caesarian section and the number of implantation sites, resorption sites, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, gross pathology incidence of the dam urogenital tract, number of implantation sites, number of corpora lutea, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported (these data were described in the robust summary for reproductive effects for the test material). The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10x magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects (the maternal and developmental fetal effects are discussed in this robust summary).
NOAEL(NOEL) maternal toxicity	600 mg/kg bw/day
NOAEL (NOEL) developmental toxicity	600 mg/kg bw/day
Actual dose received by dose level and sex	0, 6, 28, 130, or 600 mg/kg bw of the test material (FDA 71-28)
Maternal data with dose level	Daily clinical observation and measurement of body weight gain failed to show any differences between control and test groups of female rats. The number pregnant and % pregnancy were similar for all dose and control groups. No abortions were observed in any group.
Fetal Data with Dose Level	The average fetal weight of treatment and control groups were not statistically different ($p>0.05$). The total number of live fetuses were similar for test and control groups. A small % of (less than 3%) dead fetuses were observed at the three highest dose levels. Skeletal examination of sternebrae showed no significant differences in the incidence of incomplete ossification or missing sternebrae for test and control groups.

	ossification or missing sternebrae for test and control groups. Likewise the incidences of fetuses with more than 13 ribs, incomplete ossification of vertebrae and extremities, incomplete skull closures were similar for test and control animals. Visceral examination failed to reveal any evidence of abnormalities at any dose level.
Conclusion Results	There was no evidence of maternal toxicity or developmental toxicity at dose levels up to and including 600 mg/kg bw/day of test material.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Morgareidge K. (1973b) Teratologic evaluation of FDA 71-28 in hamsters. Contract No. FDA 71-260. Unpublished report.

Substance Name	Estragole
CAS No.	140-67-0
Remarks for Substance	Data is for the structurally related substance, safrole
Test Type	Developmental toxicity
GLP	No
Year	1985
Species/strain	Swiss Mice
Sex	Female
Route of Administration	Intragastric
Duration of Test	Not given
Doses/concentration Levels	0-200 mg/kg bw/d
Exposure Period	8 days (day 6-14 of pregnancy)
Frequency of Treatment	Daily
Control Group and Treatment	Not given
Remarks for Test Conditions	Safrole was administered intragastrically to female Swiss mice from days 6-14 of pregnancy.
NOAEL(NOEL) maternal toxicity	Not given
LOAEL(LOEL) maternal toxicity	Not given
NOAEL (NOEL) developmental toxicity	Not given

LOAEL (LOEL) developmental toxicity	Not given
Actual dose received by dose level and sex	Not given
Maternal data with dose level	Toxic to dams.
Fetal Data with Dose Level	No significant increase in malformations.
Appropriate statistical evaluations	Not given
Remarks for results	Article in Italian. Summary provide in English.
Conclusion Results	Safrole was not teratogenic to Swiss mice under the experimental conditions used.
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Only short abstract available.
References	Moro M.G., Ognio E., Rossi L. et al. (1985) Prenatal toxicity of safrole in laboratory animals. Riv. Tossicol. Sper. Clin. (Italy) 15/1-2 91-97.